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The Journal of Laboratory and Clinical Medicine

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ORIGINAL ARTICLES

STUDIES ON CHOLESTEROL

IV. EXPERIMENTS CONCERNING THE RELATION OF THE DIET, THE BLOOD CHOLESTEROL, AND THE "LYMPHOID DEFENSE"

BY GEORGINE LUDEN, M.D., ROCHESTER, MINN.

IN a series of experiments on myself made between June 17 and Dec. 30, 1916, I was able to observe a close relation as well as a reciprocal action between the diet, the blood cholesterol, and the lymphocytic reaction or "lymphoid defense." The term "lymphoid defense" was chosen in reference to the observations of Murphy and Morton, who found that animals on which cancer transplants did not "take" showed up to a 200 per cent increase in the lymphocyte count of their blood, while those not endowed with this natural resistance showed no such increase. When the lymphoid tissues were injured or partly destroyed by means of roentgen rays 100 per cent of "takes" could be obtained. The term "lymphoid defense" will be used in this paper to represent the combined percentages of the small and the large lymphocytes in the differential counts. The transitionals have not been included, as hematologists do not agree concerning the place that should be assigned to them in the classification of the blood cells, though they may play a part in the reaction against malignant proliferation. Grawitz considers transitionals to be the prototypes of the polymorphonuclear neutrophil leucocytes, Simon classifies them with the large lymphocytes, and Ziegler[61] looks upon them as closely allied to lymphoid myelocytoid cells.

The relation observed between the diet, the blood cholesterol, and the lymphoid defense suggested that beneficial results might be obtained from dietary measures different from those we are accustomed to take into account. The practical importance of this relation may be gathered from the following considerations:

*From the Mayo Clinic, Rochester, Minn.

Murphy and Morton state that the "lymphoid crisis" which they describe is to be considered "not merely an accompanying factor in the immune period, but essential to the process." In other words, the lymphocytic reaction appears to be one of the body's natural means of defense against malignant proliferation. This assumption seems to be corroborated by Stevens' recent observations on a reactive lymphocytosis induced by roentgen rays and followed by improvement in cases of malignant growth. The fact that "lymph gland extract" is being tried against malignant tumors and conditions marked by new growth of tissue, also tends to show that Morton and Murphy's interpretation of the lymphocytic reaction is gaining acceptance in medical circles.

The physiologic activity of cholesterol and the cholesterol content of the blood have been the object of a great number of recent investigations as regards their relation both to normal growth and to various pathologic conditions, by Robertson,[40] Browder, Luden,[28] Robertson and Burnett,[39] Rothschild,[42, 43, 44, 45] Bloor,[3, 4, 5, 6] and others. Luden[28] has called especial attention to the effect of cholesterol retention on cell proliferation.

The influence of the diet on the cholesterol content of the blood, which had been studied previously in animals because of its relation to arteriosclerosis by McMeans, Saltykow and others, or its relation to the function of diverse organs (the adrenals, Krylow, Sternberg; the spleen, Soper; the liver, Chalatow, Rothschild, and others) has lately been given a clinical application by Rothschild in his work on the dietetic management of hypercholesterinemia in cases of cholelithiasis. This work shows that dietary measures calculated to reduce the blood cholesterol will relieve the symptoms of a group of patients to whom cholecystectomy, and often secondary operations have brought only temporary relief.

So far as the writer is aware, the relation between the diet, the lymphoid defense, and the cholesterol content of the blood has never been studied, yet the deduction seems admissible that dietary measures calculated to reduce the blood cholesterol while stimulating the lymphocytic reaction might prove as beneficial in cases of carcinoma before and after operation as the regulation of the diet in cases of chronic hypercholesteremia after cholecystectomy. The good results obtained by postoperative treatment of malignant growths with radium and roentgen rays are well known, and Stevens' observations suggest that these results may be due to the lymphocytic reaction induced by the rays. In a small number of cases which the writer has had the opportunity to study, both radium and the roentgen rays appeared to increase the lymphocyte count while they lowered the cholesterol content of the blood. The latter observation was reported also by Luden[29] in connection with the effect of experimental roentgen treatment on the blood of goats. There is no reason to suppose that the activation of the lymphoid defense by dietetic measures should be less beneficial than that produced by means of radium or the roentgen rays. On the contrary, it is to be expected that therapeutic measures that keep within the bounds of normal physiologic activity, so to speak, will be less apt to tax the general health of a patient than any extraneous measures.

Before describing the experiments with which this paper is concerned, it will

be necessary to discuss three factors that were bound to exert a great influence on the results obtained; namely, the individual cholesterol standard of the blood, the cholesterol content of certain articles of food, and the effect of digestion on the cholesterol content of the blood.

THE INDIVIDUAL CHOLESTEROL STANDARD OF THE BLOOD

Rothschild[42] has pointed out that normal animals have an individual cholesterol standard, the variations of which are slight. Luden[29] was able to corroborate

TABLE I

BLOOD CHOLESTEROL OF THE WRITER, NOV. 23, 1915, TO JUNE 19, 1916*

DATE	AUTENRIETH (MG. CHOLESTEROL IN 5 C.C. CHLOROFORM)	BLOOR I (MG. CHOLESTEROL IN 6 C.C. CHLOROFORM)	BLOOR II (MG. CHOLESTEROL IN 6 C.C. CHLOROFORM)
	Autenrieth-Hellige Colorimeter		
Nov. 23, 1915	0.180		
Jan. 31, 1916		0.216	0.260
Feb. 1		0.272	0.300
Mar. 1		0.272	
9		0.272	
18		0.272	
25**		0.330	
29		0.330	
Apr. 6		0.300	
9		0.272	
17			0.330
22		0.272	
June 16		0.272	
17		0.272	0.330
	Duboscq Colorimeter		
17		0.275	0.333
18		0.272	0.333
19		0.272	0.330

*The technic used for these tests is identical with that described in Luden's[29] studies on the cholesterol content of the blood of goats, with the exception only that the Duboscq colorimeter set at 10 mm. and a standard test 0.400 mg. was used instead of the Autenrieth-Hellige instrument. It should be remembered also that all colorimetric estimations are influenced by the individual "color vision" of the operator, even when all other factors are identical; hence my "normal" cholesterol values which were found in a series of healthy persons read somewhat higher than the values quoted by Bloor.[17] It should be added that in a large series of tests run parallel with the Duboscq and the Autenrieth colorimeters the difference between the readings of the two instruments kept constantly within the following range: Duboscq 0.222, Autenrieth 0.216; Duboscq 0.275, Autenrieth 0.272, the greatest difference being 0.008 mg. Consequently the cholesterol values obtained while the Autenrieth colorimeter was still in use, do not need any correction. Details concerning these parallel tests and of cholesterol values found in health and disease will be published shortly.

**Slight attack of appendicitis.

these findings by observations on the cholesterol content of the blood of goats. Similar observations were made regarding the cholesterol value of normal persons partaking of an ordinary mixed diet. My own cholesterol standard was established by a series of determinations made between Nov. 23, 1915, and June 16, 1916 (Table I). It remained practically constant at 0.272 mg. in 6 c.c. of chloroform extract made according to Bloor's original method[3] for the determination of blood cholesterol. The only considerable exception occurred during a very slight attack of appendicitis, when the cholesterol went up to 0.330 mg. It returned to normal, however, within a fortnight.

THE CHOLESTEROL CONTENT OF FOODS

Data concerning the cholesterol content of the usual articles of food are extremely scarce, and so far as can be ascertained, no such determinations have been made by the Bloor method. For the sake of a fair comparison with the changes observed in the cholesterol percentage of the blood during any given diet it seemed important to establish the cholesterol percentage of the components of these diets by the same method by which the blood was tested. Table II shows the values found in samples of the food used during the diet experiments. It should be remembered that a number of factors are bound to play an important part in the cholesterol value of foodstuffs. The breed, the age, and the mode of feeding animals will not be without influence on the cholesterol content of the food the animals supply. Even different parts of the meat of the same animal may have slightly different values. The time of calving materially affects the richness of the milk and its percentage of cholesterol, and in the case of cheese, for instance, the special brand and the ripening may not be without effect. Absolute data can, therefore, be obtained only by an extremely large number of deter-

TABLE II

CHOLESTEROL OF FOODS

FOOD	WRITER'S DETERMINATIONS. DUBOSCQ COLORIMETER. *Bloor I* (mg. cholesterol in 6 c.c. chloroform)	*Bloor II* (mg. cholesterol in 6 c.c. chloroform)	OLDER METHODS[15] (PER CENT)	
Yolk of egg, raw,.. 3 gm	2.660	2.660	2.15	1.75
Yolk of egg, hard boiled, 49% water	2.660	2.660		
Yolk of egg, desiccated	4.000	4.000		
Milk, raw, 3 c.c	0.080	0.080		
Cream, raw, 3 c.c	0.185	0.185		
Butter, 3 gm	0.472	0.880*	0.190	
Butterfat			0.200	0.220
Maize			0.100	
Mushrooms (agaricus campestris)	0.444	0.307	0.520**	
Mushrooms (agaricus campestris) with sauce	0.210	0.181		
Beef, raw, 3 gm	0.160	0.160		
Beef, roast	0.190	0.190		
Beef, smoked, dried	0.181	0.250		
Chicken, roasted, breast	0.380	0.380		
Fish, black bass	0.250	0.250		
Oatmeal	0.000**	0.000***		

*Bloor II tests appear to contain some kind of coloring material.
**Boletus edulis.
***Autenrieth method also negative.

minations. The writer is fully aware of this, but has decided to publish the findings in the following tables for two reasons: First, the cholesterol content of the various articles of food used during the experiments must be closely associated with the results obtained; second, since present data on the subject are either vague, (Rothschild,[45] for instance, states that eggs, cream, milk, and cheese are rich in lipoids, but gives no definite values) or obtained by older methods, it may be of general interest to know the approximate cholesterol percentage of at

least a few of the common articles of food obtained by the Bloor method, allowance being made for slight variations in different samples of the same kind of food (Table II).

In selecting the sample of fish, a very dry piece from the center near the spine was used and tested with blotting paper to see that no trace of grease was present. The difference between the percentages for raw and roasted beef is explained by the loss of water that occurred in roasting. It is interesting to note that the different tests of the yolks of eggs correspond closely. The tests were made with a Duboscq colorimeter set at 10 mm. and the standard cholesterol solution containing 0.400 mg. per 6 c.c. of chloroform used in our blood cholesterol determinations.

INFLUENCE OF DIGESTION ON THE BLOOD CHOLESTEROL

Bloor[3] states that in dogs the process of digestion and the chemical constituents of the food do not influence the cholesterol content of the blood to any appreciable extent. The effect of digestion in ruminating herbivora has been discussed in my work on the blood of goats. As far as could be ascertained no data have yet been published on the changes occurring in the blood cholesterol of normal persons during the digestion of the average mixed diet.

Three experiments were made to study the effect of the digestive process on the writer's cholesterol standard during the usual mixed diet. In every instance the first test was made before breakfast, as Bloor[6] has found that the postabsorptive condition eight to sixteen hours after the last meal is "practically the only time when the blood is free from the influence of ingested or mobilized fat." According to Bloor and Rothschild, cholesterol and fat metabolism are closely associated. Whatever may be the exact time required for elimination of the other lipoids from the blood, the results obtained in these experiments seem to indicate that the blood cholesterol returns to its "standard value" in approximately four hours even when a considerable amount of food rich in cholesterol has been consumed. Consequently the time allowed—twelve hours—would be amply sufficient to exclude the effect of digestion from the first test. The subsequent tests were made at one hour's interval each, with the exception of the first test after the midday meal, which had to be taken after two hours on account of the absence of those who took the blood. For each test 3 c.c. of blood were taken from the cubital vein by means of a graduated syringe fitted with a hypodermic needle, according to Bloor's method,[3, 4] the method used in all my experiments. The blood was boiled up immediately after it had been taken. It was found necessary to let a week elapse between the two experiments as the repeated introduction of the hypodermic needle caused the cubital veins—the left and right were used alternately—to collapse, small thrombi being formed which obstructed the lumina. Attempts to use the veins on the back of the hand gave unsatisfactory results, the caliber of most of these being too small, or, when sufficiently large, it was impossible to fix the vein itself and it slid aside when the needle was introduced.

The results shown in Table III were obtained in these experiments:

As may be seen from the foregoing experiments, a light luncheon consisting of bread with butter and tea or coffee does not appear to produce any marked changes in the blood cholesterol, or at any rate these changes have been eliminated within an hour. The tests made after the more substantial meals seem to show that the cholesterol content of the blood returns to its "normal standard" in approximately four hours even when as much as three-fourths of a pound of raw beef and the yolk of an egg have been consumed at one meal. This fact should be borne in mind in considering the results obtained from an exclusively meat diet. (See Table V.)

EXPERIMENTS SHOWING THE EFFECT OF THE DIET ON THE CHOLESTEROL CONTENT AND THE CYTOLOGY OF THE BLOOD

The following experiments were suggested by the current belief that there is a causative relation between the increased consumption of meat and the incidence of cancer. Many writers do not hesitate to endorse this view. Williams writes: "I am persuaded that the ascertained facts justify the belief that there is a certain relation between the conditions of nutrition and the incidence of cancer growth." Bulkley has repeatedly called attention to the influence of the diet on the course of malignant disease. Von Müller and von Bauer[33] in their clinical lectures both emphasized the fact that the increase of cancer in Bavaria might be due, in part at least, to increased consumption of meat among the Bavarian peasantry. Hoffman comes to similar conclusions from his cancer statistics.

Cytologic changes in the blood in malignant disease have been reported by

TABLE III

HOURLY TESTS OF CHOLESTEROL IN WRITER'S BLOOD DURING THE PROCESS OF DIGESTION

FIRST EXPERIMENT, AUG. 2, 1916

Blood taken before breakfast showed a cholesterol value of 0.272 mg. by the Bloor I test and 0.333 mg. by the Bloor II test; that is, the usual interval between the two tests previously observed in the postabsorptive period.*

Breakfast (8 A.M.)
2 egg sandwiches (1 egg)
½ cantaloupe
2 cups of tea (milk and sugar)

	DUBOSCQ COLORIMETER	
	Bloor I	*Bloor II*
	(*mg. cholesterol in 6 c.c. chloroform*)	
Blood taken 1 hour after breakfast.....	0.347	0.420
" " 2 hours " "	0.333	0.380
" " 3 " " "	0.280	0.350
" " 4 " " "	0.272	0.330
Dinner (12 M.)		
1 cup cold broth		
1 "tartar" steak (raw)		
1 egg yolk		
1 small slice of bread with butter		
2 cups of coffee (cream and sugar)		
Blood taken 2 hours after dinner.....	0.343	0.472
" " 3 " " "	0.363	0.500
" " 4 " " "	0.275	0.330

*The cause of the interval between the two tests has been discussed elsewhere by the writer and can not be considered here.

TABLE III—CONTINUED

SECOND EXPERIMENT, AUG. 7, 1916

Blood taken before breakfast showed 0.272 mg. cholesterol (Bloor I test) and 0.333 mg. (Bloor II test).

Breakfast (8 A.M.)
90 gm. raw beef
1 small slice of bread with butter
½ cantaloupe
2 cups of coffee (sugar and cream)

	DUBOSCQ COLORIMETER		AUTENRIETH-HELLIGE COLORIMETER	
	Bloor I	*Bloor II*	*Bloor I*	*Bloor II*
	(mg. cholesterol in 6 c.c. chloroform)			
Blood taken 1 hour after breakfast.....	0.330.	0.338	0.358	0.342
" " 2 hours " "	0.285	0.363	0.284	0.362
" " 3 " " "	0.285	0.363	0.284	0.362
" " 4 " " "	0.272	0.333	0.272	0.330

Dinner (12 M.)
2 ham sandwiches
1 cottage cheese sandwich
1 egg sandwich (½ egg)
2 cups of coffee (sugar and cream)

Blood taken 2 hours after dinner.....	0.307	0.363
" " 3 " " "	0.272	0.333
" " 4 " " "	0.272	0.333

THIRD EXPERIMENT**

Blood taken 4 hours after last meal showed 0.272 mg. cholesterol (Bloor I test) and 0.333 mg. (Bloor II test).

Light luncheon (4-5 P.M.)
3 small slices of bread with butter
2 cups of tea (moderate amount of sugar and cream)

Blood taken 1 hour after luncheon.....	0.277	0.333

**This experiment was made several times on different dates to determine whether patients could be allowed a light breakfast, before their blood was tested.

Gruner, who based his work on the Arneth theory, and who succeeded moreover in reproducing these cytologic changes in his own blood by dietary measures.

Although some of Gruner's deductions may be open to criticism from a hematologic point of view, the main idea of his work and the honest effort which it embodied seemed to deserve recognition in a practical form; namely, the repetition of his experiment and the checking up of his results through independent investigation. Consequently certain of my experiments have been carried out along the lines suggested by him. Moreover it seemed possible that experiments of this kind might furnish data concerning the influence of the diet on the lymphocytic reaction and on the cholesterol content of the blood and, therefore, in a wider sense, on the relation of metabolism to the cancer problem.

Gruner has adopted a very detailed classification for the morphologic changes he observed in the leucocytes and lymphocytes of patients suffering from malignant conditions and in his own blood in dietetic experiments. Many of the pathologic nuclei he describes, however, somehow give the impression that they are perhaps artefacts due to accidents in staining or the mechanical effect of making the smear. Amitotic division, for instance, has been admitted for the lymphocytes and large mononuclears by Weidenreich; but some of Gruner's diagrams (for

example, Figs. 21 and 23) suggest a folding rather than an actual division of the nucleus. Two of his varieties of atypical neutrophil leucocytes, however, seem above suspicion and have, therefore, been taken into account in my experiments inasmuch as no mechanical effect could produce the peculiar shape of their nuclei. These are the "ringform" in which the nucleus is ring-like, and "bizarre forms" in which the nucleus represents a solid compact disc or bar which sometimes suggests the shape of the letter I, or J.

Hematologists (Downey, Weidenreich) consider that the "ringform" must be looked upon as an overlapping of the two ends of the nucleus since no true rings are found in human blood though they have been described in the blood of rats. They concede, nevertheless, that in perfect smears and in increased numbers these cells are striking enough to be worthy of consideration as supporting the Arneth theory.

Gruner claims that the "ringform is frequent in carcinoma," that "neutrophils with bizarre forms are characteristic for malignant conditions," that "a relative abundance of lymphocytes is not found in malignant disease," and that "one can produce the blood picture of carcinoma . . . . . by partaking of certain articles of food, notably pork and to a less extent, other red meats." These statements were corroborated in every respect by my experiments and were, moreover, illustrated in a striking manner by a case of inoperable carcinoma of the sigmoid that will be discussed later on.

My individual cholesterol standard having been established (see Table I), my individual lymphoid defense, its relation to the neutrophil count and the number of ringforms or bizarre forms present in my blood under ordinary conditions, remained to be determined. This was done by a number of differential counts. The blood smears were made before breakfast to eliminate the influence of digestive leucocytosis; 200 cells were counted from 3 smears on every occasion. The variations in the different counts were found to range from 29 to 31 per cent for the lymphoid defense and from 49 to 55 per cent for the neutrophils. The average number of atypical forms in the latter was 2 to 3 per cent of ringforms and 2.5 to 3.5 per cent of bizarre forms. For three consecutive days preceding the experiment the relation was as follows: Lymphoid defense: neutrophils : : 30 : 54; ringforms : bizarre forms : : 2 : 3.

TABLE IV

THE EFFECT OF GRUNER'S DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 4, JUNE 20-23, 1916

Gruner's diet: Milk and water, lettuce and toast; no butter, but jam *ad libitum*. Very little jam was used.

DAY	BLOOD CHOLESTEROL—DUBOSCQ COLORIMETER		LYMPHOID DEFENSE (PER CENT)	NORMAL NEUTROPHILS (PER CENT)	ATYPICAL NEUTROPHILS	
	Bloor I (*mg. cholesterol in 6 c.c. chloroform*)	*Bloor II*			RINGFORM (PER CENT)	BIZARRE (PER CENT)
1	0.342	Too brown to test	26	57	2	3
2	0.272	" " " "	30	54	1	3
3	0.186	" " " "	33	46	1.5	3

The slight initial increase observed in the blood cholesterol may have been due to the rather severe nature of the diet to which the body had to become accustomed. On the first day the writer was chronically hungry and had a severe headache in consequence, but during the two following days she felt perfectly well and comfortable. It is a well-known fact that fasting is always accompanied by an increase of the blood cholesterol (Rothschild).[44] The changes found in the cytology of the blood were still very slight, but the drop of the blood cholesterol to 0.186 mg. and the accompanying increase of lymphocytes and decrease of neutrophils is interesting when compared with the results of the subsequent experiments.

TABLE V

THE EFFECT OF AN EXCLUSIVE MEAT DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 5, JUNE 24-JULY 1, 1916

Exclusive meat diet: Three thin slices of dry bread, 4 by 4 inches, had to be taken after the third day to help the meat down.

DAY	BLOOD CHOLESTEROL—DUBOSCQ COLORIMETER		LYMPHOID DEFENSE (PER CENT)	NORMAL NEUTROPHILS (PER CENT)	ATYPICAL NEUTROPHILS RINGFORM (PER CENT)	BIZARRE (PER CENT)
	Bloor I (*mg. cholesterol in 6 c.c. chloroform*)	*Bloor II*				
1			33	54.5	0.5	1
2			28	65	2.	2
3			24	66	3.	4
4	0.342	0.388	16	72	4.5	9
5			15	73	5.5	5
6	0.358	0.416	19	70	5.5	12
7	0.374*	0.434	18	66	3.5	2
8	0.342	0.458	17	72	9	8.5

*Diarrhea.

The results obtained by the all-meat diet seem worthy of consideration for several reasons. The changes observed in the cytology and in the cholesterol content of the blood are too marked and too gradual to be explained by mere coincidence. The increase in the blood cholesterol from 0.186 mg. to 0.372 mg. can not be attributed to the effects of hunger because very considerable quantities of meat were consumed at each meal. It is to be regretted that at first the exact amount was not determined by weight, but it may suffice to state that the writer conscientiously tried to eat as much meat as she possibly could for breakfast, luncheon and dinner, a smaller quantity being ingested at 4:00 P. M. It was subsequently found that the average amount of meat consumed daily had been from 1¼ to 1½ pounds. Coffee and tea were taken as usual, but no alcohol, since it is never used. The exclusive meat diet was not accompanied by any unpleasant symptoms whatsoever, although it was found necessary to allow a very small quantity of dry bread after the third day, since meat in hot weather—the experiment was made between June 23 and July 1—loses its attractions as a steady diet. No sensation of hunger was experienced between meals as in the previous experiment, and perfect health as well as unhampered mental activity were enjoyed throughout.* On the seventh day,

*Because of its bearing on these experiments I might add that the Union Central Life Insurance Company reported most favorably on the results of their medical examination made at this time.

at the beginning of which the highest cholesterol value was registered, there was a slight attack of diarrhea. On the following day peristalsis was normal again, but the blood cholesterol had dropped to 0.342 per cent. Since the elimination of cholesterol by the feces is one of the means by which the cholesterol balance is preserved (McNee), it seems logical to assume that the body resorted to increased peristalsis in order to maintain its equilibrium of health. The fact that the same automatic regulation of the cholesterol balance was observed also in a later experiment would seem to support this view, and will be more fully discussed in connection with Experiment 7.

The gradual but steady drop in the lymphoid defense from 33 to 17 per cent, the parallel increase of the polymorphoneutrophils from 46 to 72 per cent, and the unusual number of atypical neutrophils—9 per cent ringforms, 12 per cent bizarre—which appeared in the blood as the cholesterol percentage reached its highest values, suggest a very definite relation between the chemical composition and the cytology of the blood, while the changes observed in both appear to be closely associated with the chemical nature of the food consumed. The same observations could be made in every one of my experiments.

The cytologic changes found in the blood during the exclusive meat diet are moreover in perfect accordance with Gruner's findings, which they corroborate. The two points on which he lays particular stress regarding the similarity between the blood picture during excessive meat consumption and in carcinoma—reduction of the lymphocytes and the appearance of atypical neutrophils in markedly increased numbers—seem to be borne out also by the following observations:

OBSERVATIONS A AND B

SUBJECT	BLOOD CHOLESTEROL DUBOSCQ COLORIMETER *Bloor I* (*mg. cholesterol in 6 c.c. chloroform*)	*Bloor II*	LYMPHOID DEFENSE (PER CENT)	NEUTROPHIL COUNT (PER CENT)	ATYPICAL NEUTROPHILS (RINGFORM) (PER CENT)
A. Dr. F.: Inoperable carcinoma of sigmoid	0.416	0.416	11	70	10
B. Man in perfect health, who had tropical malaria sixteen years ago	0.462	0.472	40	50	2

It is noteworthy that whereas the blood cholesterol of the healthy person is slightly higher than that of the carcinomatous patient, the unusually high lymphoid defense, 40 per cent, and the small percentage of ringforms, 2 per cent, of the former are in striking contrast to the unusually low lymphoid defense, only 11 per cent, and the high percentage of atypical neutrophils, 10 per cent, of the latter.

The relation between the blood cholesterol and the blood cytology is further illustrated by the changes produced in the blood of the same carcinomatous patient, Dr. F., by radium therapy, which temporarily arrested the disease if it did not cure it, and caused a marked improvement in the general condition.

OBSERVATIONS C AND D

SUBJECT	BLOOD CHOLESTEROL DUBOSCQ COLORIMETER *Bloor I* (*mg. cholesterol in 6 c.c. chloroform*)	*Bloor II*	LYMPHOID DEFENSE (PER CENT)	NEUTROPHIL COUNT (PER CENT)	ATYPICAL NEUTROPHILS RINGFORMS (PER CENT)	BIZARRE
C. Dr. F. after colostomy	0.330	0.374	21.5	67	3	7
D. Dr. F. after radium treatment.	0.240	0.260	33	62.5	3.5	0

During the following months the lymphoid defense of the patient dropped again to 16 per cent, which seems in accordance with the view expressed by the patient's physician, Dr. Howard Kelly, that "a permanent cure was not to be expected." It is greatly to be regretted that the blood cholesterol could not be tested again, since there is every reason to assume that a renewed increase in the blood cholesterol would have corroborated previous observations. Although at present definite conclusions would be premature, there seems little doubt that further investigations of the relation between the chemistry and the cytology of the blood may prove to be of great value in the study of malignant conditions. In the meantime the explanation seems admissible that a high lymphoid defense is effective in maintaining the normal equilibrium even in the presence of high cholesterol values, although other factors, elimination of cholesterol by the feces for instance, may be equally important. Levy and Rowntree have shown that the balance of health, that is, the chemical balance of the body, swings between extremely narrow limits. "The acidity of 'acidosis' and the alkalinity of 'alkalinosis' may be compared with perfect scientific accuracy to the 'acidity' of distilled water and the alkalinity of certain varieties of tapwater" (Rowntree). Consequently, any factors by which the normal equilibrium might be disturbed are worthy of consideration. The cholesterol content of the blood as well as the lymphoid defense would appear to be factors of no small importance.

VEGETABLE DIET

The diet used in this experiment consisted of vegetables, fruit, and dry bread or toast. No butter was used except such as had been added to the vegetables in cooking, and since the latter were not very "rich"—never creamed for instance—the amount of butter consumed can be safely considered very small. The vegetables consisted of carrots, cauliflower, string beans, asparagus, boiled or mashed potatoes, spinach, lettuce and cucumbers. In a few instances, mushrooms were not avoided, but the steady decrease of the blood cholesterol (below normal) would tend to show that their consumption did not seriously interfere with the results of the experiment even though their cholesterol content is relatively high. No fish and no meat in any form were partaken of during the time of experimentation. Coffee and tea were drunk as usual, but no alcohol in any form. It would seem that the diet was entirely sufficient to meet the requirements of the body, for the duration of the experiment at least, since no sensation of hunger between meals was experienced and no loss of weight could be observed. Peristalsis was absolutely normal and the writer felt extremely well and "fit" throughout the experiment.

TABLE VI

THE EFFECT OF A VEGETABLE DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 6, AUG. 2 TO 12, 1916						
DAY	BLOOD CHOLESTEROL DUBOSCQ COLORIMETER (*mg. cholesterol in 6 c.c. chloroform*)		LYMPHOID DEFENSE	NEUTROPHIL COUNT	ATYPICAL NEUTROPHILS RINGFORM	ATYPICAL NEUTROPHILS BIZARRE
1			17	71	3.5	3
2	0.312	0.386	28	65	0.0	3
3						
4	0.302	0.358	21	70	0.5	3.5
5			33	58	1	1.5
6	0.202	0.312	28	60	2.5	0.5
7						
8	0.242	0.312	30	61	1.5	0.5
9	0.242	0.302	29	62	1	2
10	0.242	0.302	33	57	0.5	3

The results obtained by the vegetable and fruit diet present a contrast as well as a complement to those of the exclusive meat diet. Whereas the blood cholesterol slowly but steadily decreased from 0.342 mg. to 0.202 mg. on the fifth day and finally seemed to settle down to a new standard, 0.242 mg., which was found on three consecutive days, the lymphoid defense increased from 17 per cent to 33 per cent, with a parallel drop in the neutrophils and a marked reduction of the number of atypical cells.

In order to give the body sufficient time to swing back to normal values, and because of the tendency to collapse shown by the cubital veins, described above, eleven days were allowed to elapse before another blood test was made. During this interval the usual mixed diet was used again. The following values were found at its close:

BLOOD CHOLESTEROL DUBOSCQ COLORIMETER		LYMPHOID DEFENSE	NEUTROPHIL COUNT	ATYPICAL NEUTROPHILS RINGFORM	ATYPICAL NEUTROPHILS BIZARRE
Bl or I	*Bloor II*	(PER CENT)	(PER CENT)	(PER CENT)	(PER CENT)
(*mg. cholesterol in 6 c.c. chloroform*)					
0.266	0.307	31	62	0.5	3

Apart from the very slight increase in the neutrophil count, the normal standard had been reached again. The difference between the original cholesterol standard, 0.272 mg., and the last reading, 0.266 mg., i. e., 0.006 mg., can hardly be considered significant. The intimate relation between the chemistry and the cytology of the blood and the diet, previously observed, seems to be confirmed by this experiment.

INFLUENCE OF AN EXCESS OF CARBOHYDRATES ON THE CHOLESTEROL CONTENT AND THE CYTOLOGY OF THE BLOOD

Two seemingly unrelated problems formed the basis of this experiment; namely, the cause of the epithelial proliferation on the tongues of rats observed by Stahr[55] after exclusive feeding of oats, and the ability of the body cells to synthesize cholesterol from cholesterol-free substances, defended by Dezani.[12, 13]

The practical importance of these problems and their possible relation to each other is such that a brief outline of the work referred to may be of value.

Stahr found that epithelial tumors of considerable size on the tongue originating without exception from the papillæ circumvalate could be produced in a great number of rats of different species by feeding the animals exclusively with oats. No other diet resulted in this tumor formation. Congenital tumors were never observed, although as many as seven generations of the same family were used in the experiment. The best results were obtained when the animals were fed on oats continually and almost exclusively ("dauernd und fast ausschliesslich") as soon as they had been weaned. In very old rats the excessive oat diet did not seem to cause any epithelial proliferation, while adult rats, that is, full grown but not old, animals seemed to respond to the irritant in varying degrees. Stahr attributes his results, in part at least, to chronic irritation produced by the oat husks which were found embedded in the tongues of the animals in which tumors occurred. Nevertheless, he admits that a number of wild rats in whose tongues the sharp pointed husks were also found embedded showed no trace of tumor formation. The time required for the lesions to become manifest and their respective size varied considerably in different animals. A great number of the larger tumors developed in from five to seven months, the initial stages being visible in about four weeks to two months. In many instances, however, the tumors grew so slowly that there seemed to be no visible increase after five months. Stahr considers that this divergence can be accounted for only by the fact that the rats were not endowed with the same degree of predisposition ("dass die Ratten eben verschieden stark disponiert waren"), a conclusion which will readily be admitted. He adds that our conception of the nature of this so-called "disposition und konstitution," which has been somewhat vague for many years has become clear and definite as a result of recent investigations. The accuracy of the latter statement seems questionable. That the mechanical factors which he enumerates—anatomical formation of the mouth and motility of the tongue, either of which impeded or promoted the removal of the implanted husks, the nature of the irritant itself and the length of time the irritation was kept up—played an important part in the process of atypical proliferation can not be doubted, but there are other factors the significance of which should not be underrated; namely, the chemical composition of the oat diet and the power of elimination of the individual rat. Notwithstanding the masterly analysis of causes found in his report, Stahr does not seem to take these factors into consideration, for the internal causes (innere Ursachen) which he discusses at great length are of an entirely different character. That both the "disposition and constitution" to which he refers, however, are intimately associated with the ability of the individual organism to eliminate or metabolize certain articles of food, will, I think, be readily conceded. Many of the problems of individual metabolism have by no means been solved as yet.

In discussing the nature of the growths produced by the oat diet, Stahr states that they represent "at least the earliest evidence of an epithelial tumor, of a true blastoma,"[55] (page 225). He discusses the relation between harmless proliferation and malignant hyperplasia at great length and comes to the con-

clusion that in the initial stages it is impossible to decide whether any given type of atypical epithelial proliferation is destined to retain its benign character or to develop into a true carcinoma, "so that consideration must be taken of internal causes which affect these originally benign growths in such a way that they discard normal bounds and develop into cancer." The context shows that Stahr evidently looks for the "internal causes" within the tumor cells themselves, inasmuch as he defends the hypothesis that in malignant conditions new types of cells are formed in circumscribed areas, and lawless proliferation is an inherent characteristic of these newly formed cells. In other words, the tumor cells as such are responsible for their onslaught on the welfare of the body. Would it not be equally reasonable to assume that lawless proliferation is the manifestation of "internal causes" in another sense; that is, that some substance stimulating cell proliferation is supplied by the body itself through faulty metabolism, and that the atypical character of these proliferating cells is merely the result of an overhurried rate of cell-production under the influence of this constantly applied stimulant? We know that in hyperthyroidism, for instance, atoxic substance is constantly produced which goads the heart into lawless activity, and that infinitesimal doses of this substance, Kendall's[26] alpha iodine compound, similarly increase the heart rate of normal animals for a short time. This has been demonstrated experimentally. It has also been found that the normal heart swings back to its usual rate of action as soon as the toxic compound has been either eliminated or destroyed by normal metabolism. We admit the "unripe" character of the so-called malignant cells and we know that the more embryonic or unfinished the cells of a tumor are, the more rapid and destructive its growth. Would it not be reasonable to deduce from the foregoing facts, that substances supplied in the food and insufficiently metabolized by inadequate organs could become the cause of lawless cell proliferation, inasmuch as the daily intake of food would furnish a constant stimulant that might bring about the hurried coinage of unfinished, atypical cells, embryonic in character, simply because the rate of production did not allow them time to become full-grown? It is well known that under suitable conditions normal cells can be transplanted and made to grow *in vitro* (Foote[16] and Burrows). The origin of metastatic tumors can be traced in many instances to strands of single cells that have been crowded out by the rapid growth of the mother tumor, and the danger of scattering and implanting stray tumor cells in operative procedures is recognized by surgeons. These observations, as well as many others that can not all be enumerated here, seem to support the view that the body itself furnishes the conditions by which proliferation is either regulated and kept within normal bounds or incited to become lawless and destructive. That the invasive growth of tumor cells ("infiltratives Wachstum," Joest and Ernesti) might be interpreted as merely the result of the increased rate of production referred to above, does not seem inadmissible.

The fact that the majority of the oat-fed rats developed tumors, the striking absence of tumors in animals fed on other food, and the presence of oat husks in the tongues of wild rats that showed no tendency to tumor formation (proving that the irritant alone was insufficient to produce the lesions) would seem to indicate that the diet itself and the way in which the animal was able to handle it,

may have been primary factors in the results obtained by Stahr. The chemical analysis of the blood of these tumor rats and the study of their blood cytology would undoubtedly have furnished valuable data concerning the effect of the diet on the development of the tumors.

The synthesis of cholesterol by the body cells from substances containing only the elements carbon, hydrogen and oxygen, of which the cholesterol molecule, $C_{27}H_{46}O$, is composed, has been a subject of much controversy. Bloor[6] (page 581) makes the guarded statement that together with other lipoids, cholesterol is "probably" synthesized by the animal organism, whereas Rothschild[41] (page 233) boldly asserts that "there is no synthesis of cholesterol in the body." In discussing the question, Bloor calls attention to the work of Dezani,[12, 13] Gardner and Lander. While the experiments of Gardner and Lander furnish a striking illustration of the power possessed by the animal organism to transform cholesterol into body cells, or at any rate, to utilize it for the purpose of cell formation, the term "synthesis" would hardly seem applicable to the process they studied; that is, the gradual development of the chick embryo from the egg. Synthesis may be defined as "the artificial building up of a chemical compound by union of its elements." Since the egg already contains a high percentage of cholesterol, the growing chick does not need to effect the union of the elements but merely converts the substance into cells. Moreover, "the fully developed chick contains only as much cholesterol as the egg did before hatching" (Rothschild,[41] page 230).

Dezani's work, on the other hand, seems far more suited to demonstrate a true synthesis of cholesterol, as a brief abstract of his original report will show. Having established the average weight and the average total cholesterol content of a certain breed of mice, he divided the sixteen remaining animals into groups of four. These animals were fed for twenty-three days on a mixture of casein and maize from which all the cholesterol had been extracted for a week with ether-alcohol. In addition, Group I received a certain amount of mineral salts, Group II mineral salts and lecithin, Group III mineral salts and fat in the form of fatty acids and glycerin, and Group IV mineral salts, lecithin and fat in the same form. The mice devoured this food greedily and seemed to thrive on it. Young growing mice had been chosen for the experiment. They gained in weight, developed normally and seemed in perfect health. At the end of seventeen days however, they began to dislike the food and lost slightly in weight. Their coats became rough, but they were lively and the ears showed no sign of anemia. On the twenty-third day, three of the mice were found dead in their cage and the rest were killed. The total cholesterol of each group was then determined by desiccating the bodies *in toto* and extracting the cholesterol by means of a special procedure devised by Dezani. Though no cholesterol had been supplied in the food, chemical analysis had shown that cholesterol had been daily eliminated by the feces, and the postmortem determinations proved that the total average cholesterol had been increased by about one-third in each group. The mice had been weighed in groups, and every group exceeded its initial weight, whereas a slight loss of weight which occurred during the last days of the experiment did not exceed 0.8 per cent of the total gain. Dezani considers, therefore, that the

destruction of body cells alone can not account for the marked increase of the total cholesterol, though it may have added slightly to the values, and that the animals must have synthesized the greater part of the surplus cholesterol from the cholesterol-free food on which they had been fed. He suggests the following explanation:

Under ordinary circumstances the body does not synthesize cholesterol, the amount essential to health being easily obtained "ready-made" in the food. Under special conditions, however, when no cholesterol is available, synthesis is resorted to by the body as an extreme measure in preference to the alternative of a cholesterol deficit. In other words, the body can synthesize cholesterol under the stress of necessity just as the heart can use all its reserve power when occasion demands. The organism soon tires of the unwonted effort, however, and loss of health, or decompensation, results in the end, as was the case with Dezani's mice.

The question suggested by Stahr's experiment is the following: Could the results obtained have been due to a reduction of the lymphoid defense with parallel increase of the blood cholesterol in those animals which responded to the excessive oat diet by tumor formation? The relation between the blood cholesterol, the lymphoid defense, and the diet observed in the writer's experiments and the part played by cholesterol in cell proliferation both in normal growth and in the growth of tumors (Robertson and Burnett), justify this supposition, especially as Stahr's report contains no reference to the blood cytology or blood chemistry of his animals.

No data being available in regard to the cholesterol content of oats, and the only chemical analysis of any kind of grain recorded being that of maize with 0.10 per cent of cholesterol (see Table I), it was decided to make a test by the Bloor method of the broken oats sold under the name of "Scotch oatmeal."* This test was entirely negative. A second test was made according to the Autenrieth method on the assumption that the Bloor test might not be effective in dissociating certain chemical compounds in which the cholesterol in oats might be bound up, though as Mueller[35] has pointed out, Bloor's method of extraction "will be found to be practically complete" in other substances. By Autenrieth's method the substance to be tested is boiled for two hours with 25 per cent potassium hydroxide, and the combined action of strong alkali and boiling could reasonably be expected to effect the necessary dissociation if any cholesterol were contained in the oats in compound form. However, the results of this test were negative also. Since, then, no cholesterol appeared to be contained in oats, and Dezani's work suggested that even cholesterol-free food can cause an increase of the total cholesterol content of the body by synthesis of cholesterol, it still seemed possible that Stahr's rats had synthetized their cholesterol from the cholesterol-free oats. This surmise did not seem unreasonable because, as they are carbohydrates, oats contain the very elements, carbon, hydrogen, and oxygen, of which the cholesterol molecule is composed.

In order to verify this supposition a diet consisting as exclusively as possible

*Scotch oatmeal consists entirely of broken oats, and should not be confounded with the so-called "rolled oats."

of broken oats (Scotch oatmeal) was decided upon. Such a diet, even though the husks had been removed from the grain, would contain practically the same chemical compounds as the oats on which Stahr's rats had been fed. The chemical effects of the diet on the cholesterol content and the cytology of the blood being the chief object of investigation, the absence of the oat husks did not seem of great practical importance. That no vitamines were lost by the removal of the oat husks would seem to be demonstrated by Fig. 5, which shows that the oat grains were practically intact and surrounded by a slightly hairy capsule, thus being comparable to thrashed but unpolished rice.

Although for reasons to be discussed later, this experiment did not furnish conclusive data concerning the synthesis of cholesterol in the body, the results obtained may be of value inasmuch as they again confirm previous observations regarding the intimate relation between the diet, the lymphoid defense, and the cholesterol content of the blood.

Rothschild[44] has pointed out that the cholesterol content of the blood is increased by starvation, cholesterol being liberated from the body fat in which it is stored. Loss of weight was, therefore, to be studiously avoided during the oatmeal diet. It might be argued that the maintenance of the original body weight and especially an increase thereof in the course of the experiment would be ample proof that any rise in the blood cholesterol could not be attributed to the destruction of body tissues. However, Dezani's[12] first experiment had shown that the body will make every effort within its power to maintain its cholesterol balance even during starvation, and that a loss of 41 per cent of body weight is accompanied by a loss of only 15 per cent of the total cholesterol. Consequently, it seemed safer not to depend on the evidence of body weight alone, but to base the experiment on a strict maintenance of the metabolic balance in general. The following standards were therefore established:

1. The diet must consist of ground oats (Scotch oatmeal) as exclusively as possible.

2. It must contain all the elements needed to maintain the metabolic balance; namely, the requisite number of calories, the relative amounts of protein, fat, and carbohydrates.

3. The food requirements, compiled from Friedenwald and Ruhräh's[18] (pages 48-79) data corresponding to the age, sex and occupation of the experimenter, must be met, if necessary, by additional foodstuffs containing as little cholesterol as possible.

According to Voit, Rubner, Tigerstedt, Atwater and Benedict,[18] quoted by Ruhräh,[18] (pages 51-54), the daily food requirements of the male adult doing light and moderately light work are as follows:

	CALORIES	PROTEIN GM.	FAT GM.	CARBOHYDRATES GM.
Voit: Physician at moderate work,	2,833	127	89	362
Rubner: Male adult at moderate work,	2,600			
Tigerstedt: Shoemaker at moderate work,	2,001-2,400			
Atwater: German physician,	2,680	131	95	327
Japanese professor,	2,380	123	21	416

Friedenwald and Ruhräh[18] (page 69) suggest the following standard:

For light work, 17 calories per pound of body weight.
For moderate work, 20 calories per pound of body weight.

They state further (pages 55-56) that "curiously enough, mental work does not apparently utilize heat or energy in the ordinary way. . . . . In a respiration colorimeter, hard mental work; i. e., the working out of abstruse mathematical problems requiring hours of time, does not cause any difference in registration. The same apparatus, however, is sufficiently sensitive to register the heat generated by turning over in bed or by raising an arm." In discussing the relation of sex and body weight to food values required, they state[18] (page 57): "On an average, women are only about four-fifths as large as men, and consequently dietaries for groups of women will require four-fifths the amount of food."

In consideration of these data and since carbohydrates were to form the chief component of the oatmeal diet, the food requirement of the Japanese professor given by Atwater, divided by four-fifths, were used as the basis for the oatmeal experiment.

EXPERIMENTER	OCCUPATION	CALORIES	FOOD REQUIREMENTS PROTEIN GM.	FAT GM.	CARBOHYDRATES GM.
40 years old; Weight, 134.5 lb.	Laboratory research, Mental, light manual,	2,000	100	30	325

The amount of fat was increased somewhat because the climate is rigorous, the experiment being made at the beginning of the cold season between October 26 and November 1, and because a liberal margin of fat in the diet might also guard against the combustion of the body fat. Moreover, the values quoted above would coincide satisfactorily with Ruhräh's estimate of the food requirements of a woman weighing 134.5 pounds at light work—17 (calories per pound of body weight, man at light work) x 134.5 (pounds body weight) ÷ $\frac{4}{5}$—and the relative caloric values given by him for protein, fat and carbohydrates:

1 gram protein	= 4 calories	100 grams protein	= 400 calories
1 " fat	= 9 "	30 " fat	= 270 "
1 " carbohydrates	= 4 "	332 " carbohydrates	= 1328 "
		Total.....	1998 "

From the figures given by Friedenwald and Ruhräh[18] (page 49), the fuel value of oatmeal and its relative percentage of protein, fat, and carbohydrates may be calculated as follows:

	CALORIES	PROTEIN	FAT	CARBOHYDRATES
Oatmeal (raw) 1 lb.	1800*	67 gm. (15%)	22 gm. (5%)	360 gm. (80%)

*Rolled oats = 1850.

Consequently the consumption of one pound of oatmeal would almost suffice to meet the food requirements, the slight deficit in the calories and protein being easily balanced by the addition of a small amount of other food with low cholesterol content, while the surplus of carbohydrates (28 gm.) contained in the oatmeal made up the deficit in fat. (2.5 gm. of carbohydrate being equivalent to 1 gm. of fat.)

An unexpected difficulty presented itself, however, in the process of cooking, owing to the amount of water absorbed by the cereal, the original bulk of oatmeal increases no less than four times. As a result, the consumption of a whole pound of oatmeal in its magnified form proved to be a physical impossibility, defying the most determined efforts. A compromise had to be resorted to and the following diet was adopted:

	CALORIES	PROTEIN GM.	FAT GM.	CARBOHYDRATES GM.
Oatmeal, ¾ lb. (raw weight)	1200	48	16	240
Oranges, 3	180	0	0	18
Milk, 2 glasses	320	14	24	30
Sugar, ¼ lb.	450	0	0	112
Dried beef, ⅛ lb.	66	16	3	0
	2216	78	33	400

In this combination, the total minimum requirement in calories was slightly exceeded, while the carbohydrate surplus of 100 gm. amply made up for the protein deficit of 22 gm., since according to Rubner, 232 gm. of carbohydrates represent a fuel value equivalent to 211 gm. of protein. The diet seemed to guarantee a perfect maintenance of the metabolic balance, whereas the amount of cholesterol contained in 2 glasses of milk and ⅛ lb. of dried beef (the latter chosen on account of the salts and relatively small bulk) was far below that provided in eggs, butter, and meat during the ordinary mixed diet. (Milk 0.080 mg. cholesterol; dried beef 0.181 mg. cholesterol. (See Table II.)

The following results were obtained on the six days of oatmeal diet: Within three days the blood cholesterol rose from 0.266 mg. to 0.400 mg.; simultaneously the lymphoid defense dropped from 31 to 24 per cent (Table VII). As in previous experiments, the cytology of the blood kept pace with these changes, for as the blood cholesterol reached its maximum and the lymphoid defense its minimum, the ringform and bizarre forms of neutrophil leucocytes increased from 2 and 3 per cent respectively to 8 and 12 per cent.* A steady increase in weight accompanied the above changes, showing that they were in no wise due to insufficient nutrition. On the fifth day of the experiment there was a sudden rise in the lymphocyte count from 24 to 31 per cent, preceded by a peculiar sharp pain in the left side the day before. This pain was confined to the region of the spleen. It differed from the pain caused by flatulence inasmuch as starting at 4 o'clock P. M., it never shifted or abated until about 11:30 P. M. when the experimenter fell asleep. During the two following days the splenic region remained very sore but the pain was no longer acute. The fact that the sudden increase in the lymphocyte count was preceded by pain in the region of the spleen suggests that an increased activity of that organ may have been accompanied by a certain amount of swelling. The pain itself was caused in all probability by adhesions around the spleen due to attacks of "Dutch malaria"† from which the writer suffered for

*It will be remembered, that all the data, blood counts, cholesterol values, and weight were taken before breakfast throughout these experiments and that consequently digestive leucocytosis can be eliminated as a cause of the sudden change in the lymphocyte count.

†"Dutch malaria" is clinically known as recurrent fever. The cause of the illness, the spirilla obermeieri, was found in the writer's blood at the time. Although the disease can be differentiated bacteriologically from other types of malaria, the subjective symptoms are practically identical. The spleen and the liver are often found enlarged, but these findings are by no means constant, and they did not occur in the writer's case. During one attack, however, a severe throbbing pain was felt in the splenic region and lasted for several weeks. This may have been caused by perisplenitis or by a splenic abscess. Since in the absence of a marked enlargement of the spleen neither of these conditions can be diagnosed with certainty in the living, the autopsy report alone can give conclusive evidence.

TABLE VII

THE EFFECT OF AN OATMEAL DIET AND A MIXED DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 7, OCT. 27 TO NOV. 8, 1916

DAY	DATE	FOOD	WEIGHT (LB.)	BLOOD CHOLESTEROL DUBOSCQ COLORIMETER *Bloor I* *Bloor II* (*mg. cholesterol in 6 c.c. chloroform*)		LYMPHOID DEFENSE (PER CENT)	NORMAL NEUTROPHILS (PER CENT)	ATYFICAL NEUTROPHILS RINGFORM (PER CENT)	BIZARRE (PER CENT)	REMARKS
1	Oct. 27	Oatmeal diet	134.5	0.266	0.347	31	61	3	4	Mixed diet since August; blood taken before breakfast.
2	28	"	135	0.333	0.363	32.5	54.5	5.5	4	Slight discomfort from flatulence; bowels regular.
3	29	"	135							Much discomfort from flatulence, otherwise well. Bowels regular, but stool deep yellow; no diarrhea.
4	30	"	135.5	0.400	0.420	24	65.5	6.5	5	Flatulence very pronounced from 4 P.M. till asleep, 11 P.M.; pain in spleen;* bowels and stool like day before.
5	31	"	136	0.400	0.420	31	62	6	5	Flatulence pronounced from 4 P.M. on; spleen region sore; slight diarrhea.
6	Nov. 1	"	136	0.233	0.285	26.5	62.5	6	7	Flatulence from 4 P.M. very pronounced; spleen pain slight; slight diarrhea.
1	2**	Mixed diet	136.5	0.200	0.266	31	58	3 5	3	No flatulence; bowels regular; stool deep yellow; no spleen pain; fagged; otherwise well.
2	3	"	136	0.266	0.333	36	57	1.5	2	Feeling very well; no flatulence; bowels regular.
3	4	"	135.5	0.295	0.420	27	62	5	2.5	Very well; bowels regular; stools deep yellow.
4	5					26	64			Feeling very well but slight diarrhea.
5	6	"	135	0.347	0.420	30	55	***		Feeling very well; bowels regular; stools normal.
6	7	"	134.5	0.266	0.380	30	53			Feeling well; bowels regular; stools normal.
7	8	"	134.5	0.266	0.350					

*The pain in the region of the spleen did not shift at all, thereby proving that it was not gas pain due to flatulence, as seemed probable at first. The possible origin of this pain is considered in the discussion of the experiment.

**From Nov. 1 to 2 only ½ lb. of oatmeal and ⅛ lb. of sugar were consumed, owing to the fact that the writer attended a dinner party. The low value of the blood cholesterol on the following morning was probably due to increased peristalsis during the previous day, and seems to indicate that the small amount of meat eaten at the party was not sufficient to increase the cholesterol value.

***Slides thrown away by mistake so that the average could not be established as on the preceding days by counts made by three different workers whose results tallied with only very slight differences.

several years, but of which no recurrence had been observed during the past six years.

The delicate and automatic self-adjustment by which the normal balance is regulated in health is again illustrated by this experiment. After the blood cholesterol had reached the maximum of 0.400 mg., the lymphocytic reaction alone seems to have been unable to restore the balance and the blood cholesterol was again reduced by increased peristalsis as in the "all meat" diet. The cause of the increased peristalsis will be considered in the discussion. As soon as the ordinary mixed diet was used the increase in weight, from 134.5 lb. to 136.5 lb., also disappeared and the normal weight, 134.5 lb., which had been constant for several years, was again registered within six days. The gradual loss of weight increase was accompanied by a slight rise of the blood cholesterol (0.266 mg., 0.295 mg., 0.347 mg.), showing, that although the increase in weight may have been due in part to the absorption of water from the oatmeal diet by the tissues, a certain amount of fat had also been produced, as reduction of fat is accompanied by a rise in the blood cholesterol (Aschoff, Rothschild). After the fifth and sixth days, the normal cholesterol value, 0.266 mg., remained constant. The lymphoid defense was still slightly below the figure observed at the beginning of the experiment, but the numbers of atypical neutrophils, ringforms and bizarre forms had returned to 3.5 and 3 per cent respectively.

At the time of writing, Dec. 30, 1916, the blood cholesterol, lymphoid defense, atypical neutrophils and weight are as follows, the usual diet having been eaten since the end of the experiment: Blood cholesterol 0.275 mg.; lymphoid defense, 32 per cent; atypical neutrophils, ringforms, 1.5 per cent, bizarre forms, 2.5 per cent; weight, 135.0 lb. These figures seem to support the deduction that, under normal conditions there is a constant relation between the above named factors.

DISCUSSION

Whereas the influence of the diet on the blood cholesterol and the cytology of the blood seems sufficiently clear in our experiments to require no further comment, three other points must be briefly discussed; namely, the cause of the increase in the blood cholesterol during the oatmeal diet which contained less cholesterol than the ordinary mixed diet, the cause of the slight attacks of diarrhea, which occurred whenever the cholesterol values reached their maximum, and the significance of the atypical neutrophils.

It has already been stated that the increase of the blood cholesterol in the oatmeal experiment did not furnish conclusive evidence as regards the synthesis of cholesterol from cholesterol-free food, even though Dezani's work seemed to prove that cholesterol can be synthetized under special conditions. The lack of definite proof in our case may be readily explained by the fact that the methods used by Dezani for the determination of the total cholesterol of his mice could not be applied in the writer's work for obvious reasons. As the increase of the cholesterol values could be ascertained in the writer's experiments only by means

of small blood samples, the possibility remained that cholesterol stored in various organs, the adrenals and the liver, for instance, had been mobilized in some way by the diet, and that the increase was due to a mobilization rather than to an actual synthesis. Although this deduction seemed plausible, it could not be verified, of course, and the rise of the blood cholesterol on a cholesterol-poor food was by no means explained. Mueller's[34] recent investigations may furnish a satisfactory explanation, however, inasmuch as he was able to show that the pancreas plays a hitherto unsuspected part in cholesterol metabolism. Although it had been known for some time that high cholesterol values are by no means rare, if not constant, in diabetes, the observation had not been accounted for so far as the writer is aware. Mueller[34] analyzed the gastroduodenal content of dogs after cholesterol feeding and studied cholesterol absorption in the digestive tract after elimination of the bile and pancreatic secretion (biliary fistula, ligation or resection of the pancreatic duct. He also studied the effect of the digestive enzymes on cholesterol *in vitro* and found that the pancreatic secretion was needed for the formation of cholesterol esters; that no cholesterol could be absorbed by the intestine without esterification; that no cholesterol absorption took place after ligation of the pancreatic duct; and that biliary fistulas lessened, but did not prevent the absorption of cholesterol, (the latter observation is in contradiction to Rothschild's findings),[41] but that the formation of cholesterol esters was markedly accelerated by the combined action of the bile and the pancreatic juice.

The bearing of Mueller's investigations on our experiment appear to be as follows: It seems possible that as the amount of carbohydrates provided in the oatmeal diet was relatively greater than the amount usually consumed in the mixed diet, (though it did not greatly exceed the average carbohydrate requirements suggested by Friedenwald and Ruhräh), the increased demands on carbohydrate metabolism, and especially the sudden change of diet, may have somewhat overtaxed the pancreas. If this deduction be correct, the greater part of the pancreatic secretion may have been used to metabolize the sudden increase of carbohydrates and but little of its activity could have been devoted to the esterification of the body cholesterol. Since free (nonesterized) cholesterol can not be absorbed by the intestinal mucosa, it would have remained in the circulation, causing a rise in the blood cholesterol. It is to be regretted that Mueller's[34] publication was not available at the time of our experiment and that consequently the effect of the oatmeal diet on the pancreas was not taken into consideration. Although urinalysis had given normal results both during the oatmeal diet and in the previous experiments, it is possible that blood-sugar determinations might have revealed a slight degree of glykemia and thus also have corroborated the above deduction.

The slight attacks of diarrhea which occurred in the experiments here reported whenever the blood cholesterol reached a given maximum, have been interpreted by the writer as an evidence of the automatic regulation of the cholesterol balance. This interpretation may be defended on the following grounds: Although an exclusive diet of fruit and vegetables is known to stimulate the activity of the intestinal mucosa and to increase peristalsis, the bowel

movements were absolutely normal during the experimental fruit and vegetable diet. At the same time, the blood cholesterol dropped below normal whereas the lymphoid defense was only slightly higher than usual. The writer's observations on the prompt readjustment of the cholesterol values after digestion also suggest that a healthy organism has natural means at its disposal by which it can and does prevent the accumulation of cholesterol in the blood. In relation

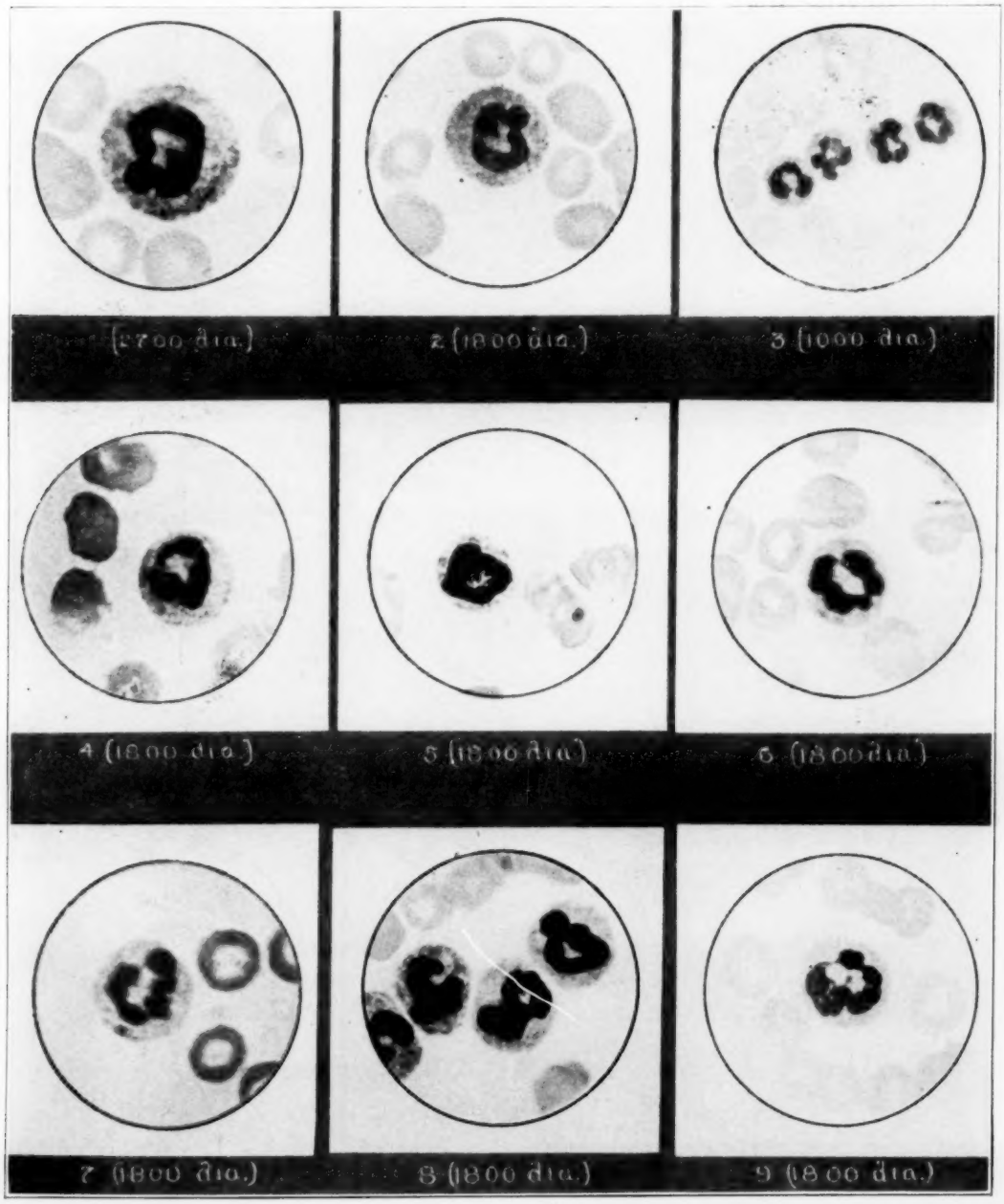

Fig. 1.—Atypical neutrophils in the blood in carcinoma of the sigmoid: Ringforms.

to this normal automatic regulation of the cholesterol balance, as well as the increase of the blood cholesterol found in a great many carcinoma cases, and the effect of cholesterol on cell proliferation referred to at the beginning of this paper, the almost constant reference to "chronic constipation alternating with diarrhea" which the writer has observed in the clinical histories of a great

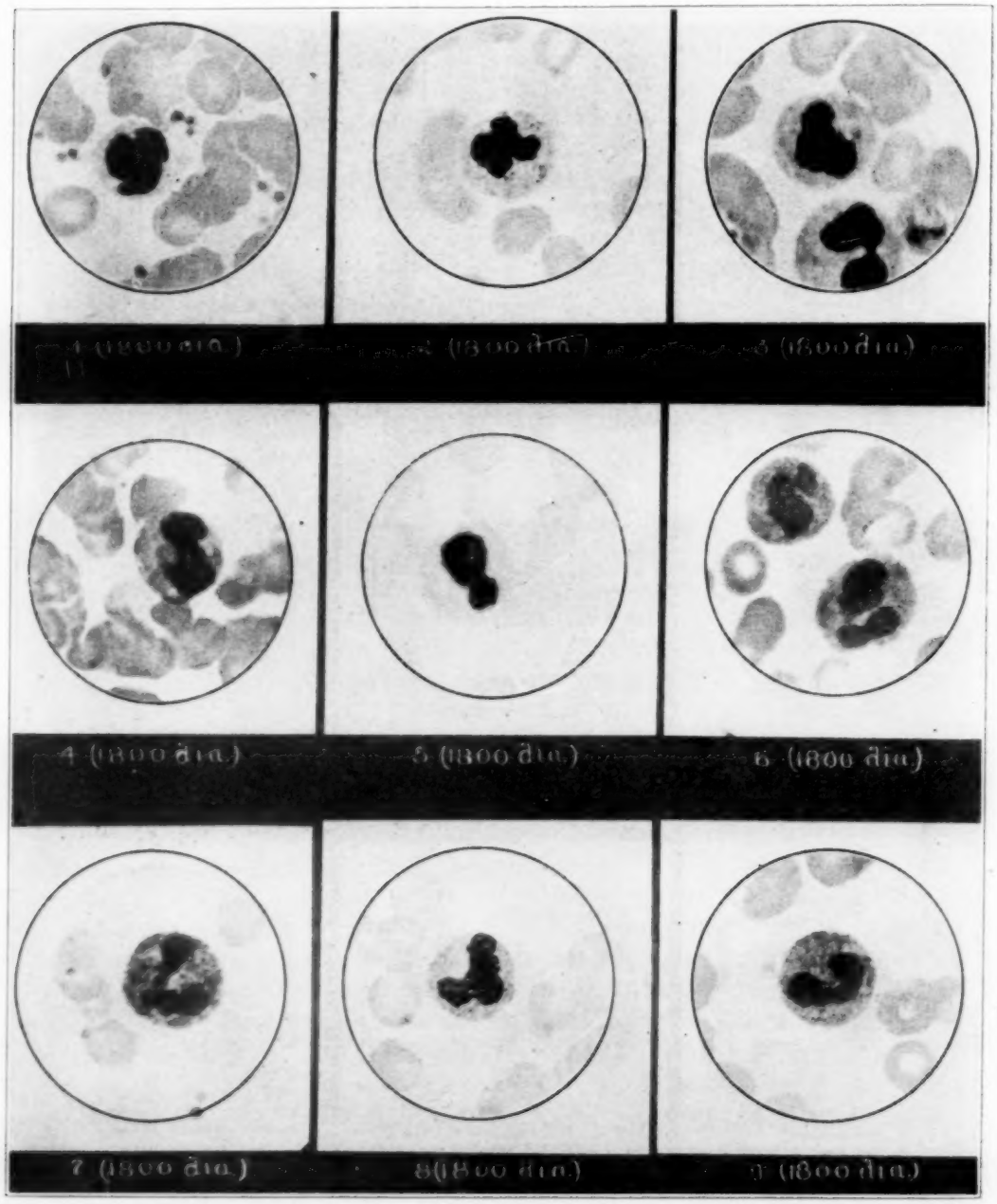

Fig. 2.—Atypical neutrophils in carcinoma of the sigmoid: Bizarre forms.

number of patients suffering from various types of cancer, may not be without significance.

The significance of the atypical neutrophils is still a matter of speculation. Their unusual, often fantastic, morphology seems to make them worth consider-

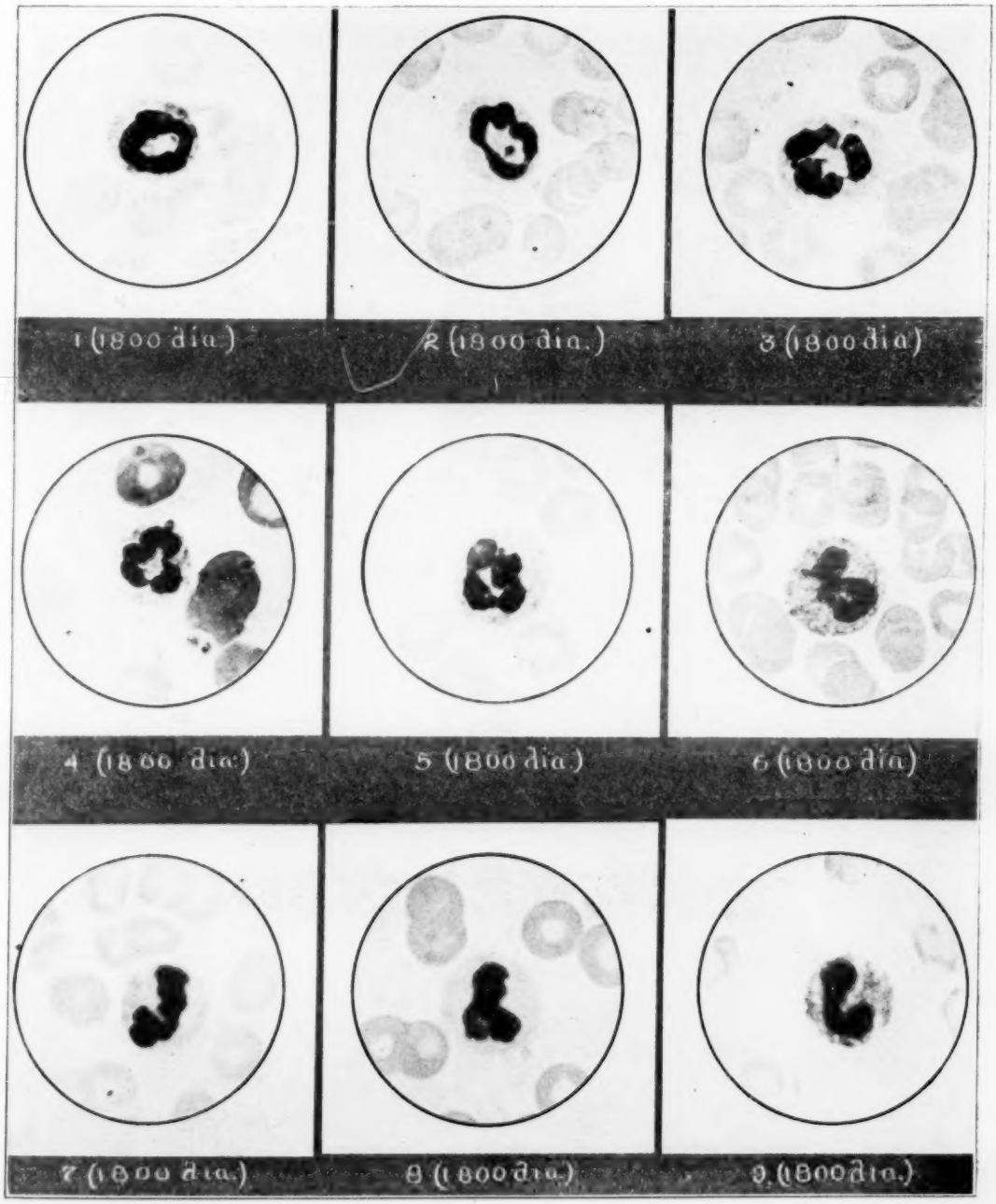

Fig. 3.—Atypical neutrophils in the blood of the writer during exclusive meat diet. (June 27, 1916.) Ringforms, 4.5 per cent: Bizarre forms, 9 per cent.

ing. The increase of their numbers in carcinoma, and under certain dietary conditions reported by Gruner and corroborated in the writer's findings, may be important, especially as in the latter instance their increase appeared to accompany the increase of the blood cholesterol. The assumption that their peculiar morphology may be in some way connected with the chemistry of the blood would seem to be supported by Weidenreich's reference to internal causes (conditions of nutrition?) in his classic work on the morphologic evolution of the nucleus in the polymorphonuclear leucocyte. Weidenreich states emphatically that the configuration of the nucleus in the "finely granular" (feinkörnig)

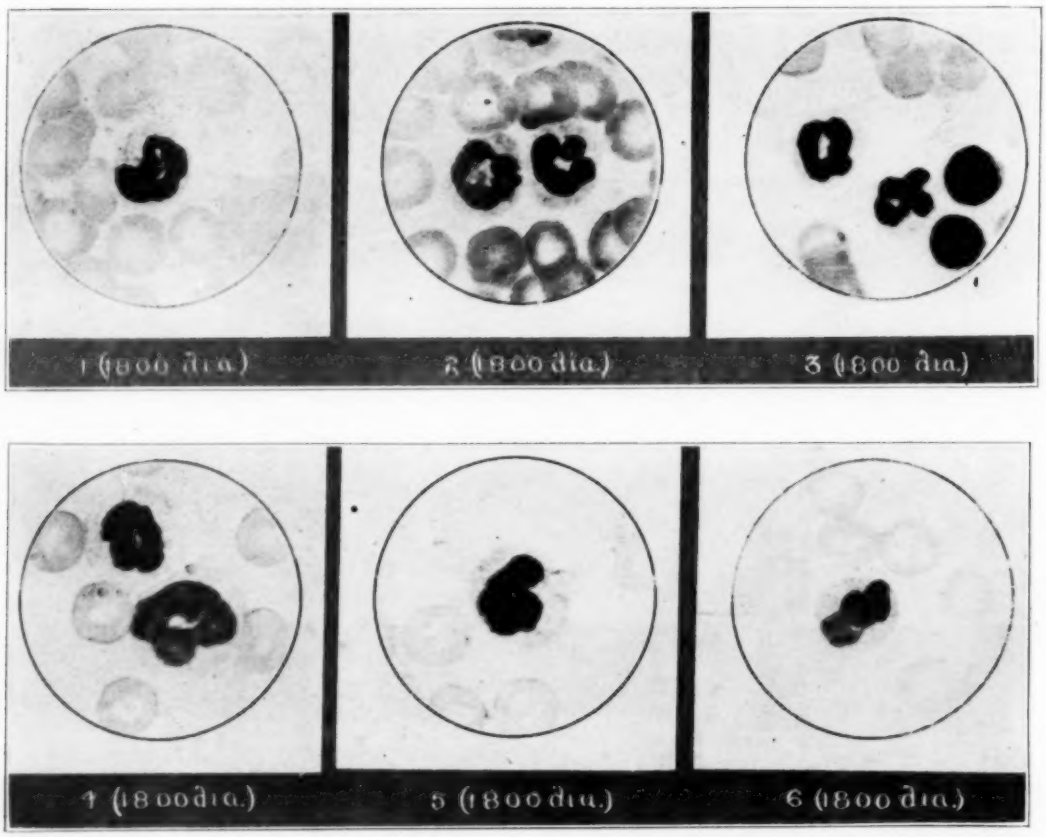

Fig. 4.—Atypical neutrophils in the writer's blood during exclusive meat diet. (June 28, 1916.) Ringforms, 5.6 per cent: Bizarre forms, 5.0 per cent. No. 4, large lymphocyte, lower field.

polymorphonuclears is due to internal causes, as well as to the morphologic differentiation of the nucleus itself. He considers that in perfect specimens the varied shapes of the nuclei are "neither artefacts nor instantaneous pictures of some phase of the motility of the protoplasm (". . . . dass die gelappten Kerne der feinkörnigen Leukocyten weder Kunstprodukte noch Augenblicksbilder einer durch die Protoplasmabewegung ständig ummodellierbaren Krenmasse ohne prazisem Formkarakter darstellen.") (p. 239). Only perfect cells that show no trace of crushing or friction have been included in the writer's counts, as may

be seen from the photomicrographs, Figs, 1, 2, 3, 4, 6, and 7. Weidenreich looks upon the more compact nuclei, solid irregular disks, Fig. 2, No. 1, for instance (the solid bars, J and L shapes, Fig. 1, Nos. 7, 8 and 9, may perhaps also come under this heading) as "juvenile forms" (Jugendformen), that will pass through various stages of morphologic differentiation until they present the well-known picture of the fragmented nucleus the component parts of which are united by

Fig. 5.—Scotch oatmeal: Broken oats. Observe that the husks alone have been removed and the slightly hairy capsule is still visible on many of the grains. (Approximately 10 diameters.)

slender threads. The relative position of the fragments may be influenced to a certain extent by the motility of the protoplasm, according to Weidenreich, but this motility plays only a secondary part in the morphology of the nucleus. That any circular arrangement of the nuclear fragments is due to the presence of a "central body" (Centralkorper) he considers "out of the question." True rings and figure-of-eight shapes are found in the blood of rats,[58] (page 226). In human blood the overlapping of the ends of the nuclear thread may produce

similar shapes, but these should be looked upon as "pseudo-rings." This overlapping can be clearly seen in several of the writer's photomicrographs (Fig. 1, Nos. 4, 8, 9; Fig. 3, No. 6; Fig. 4, Nos. 1, 5, 6; Fig. 7, Nos. 1, 2) though in many instances the process of overlapping can hardly be traced (Fig. 1, Nos. 1, 5; Fig. 3, Nos. 1, 2, 3, 4; Fig. 4, No. 1; Fig. 6, No. 2). Two neutrophils

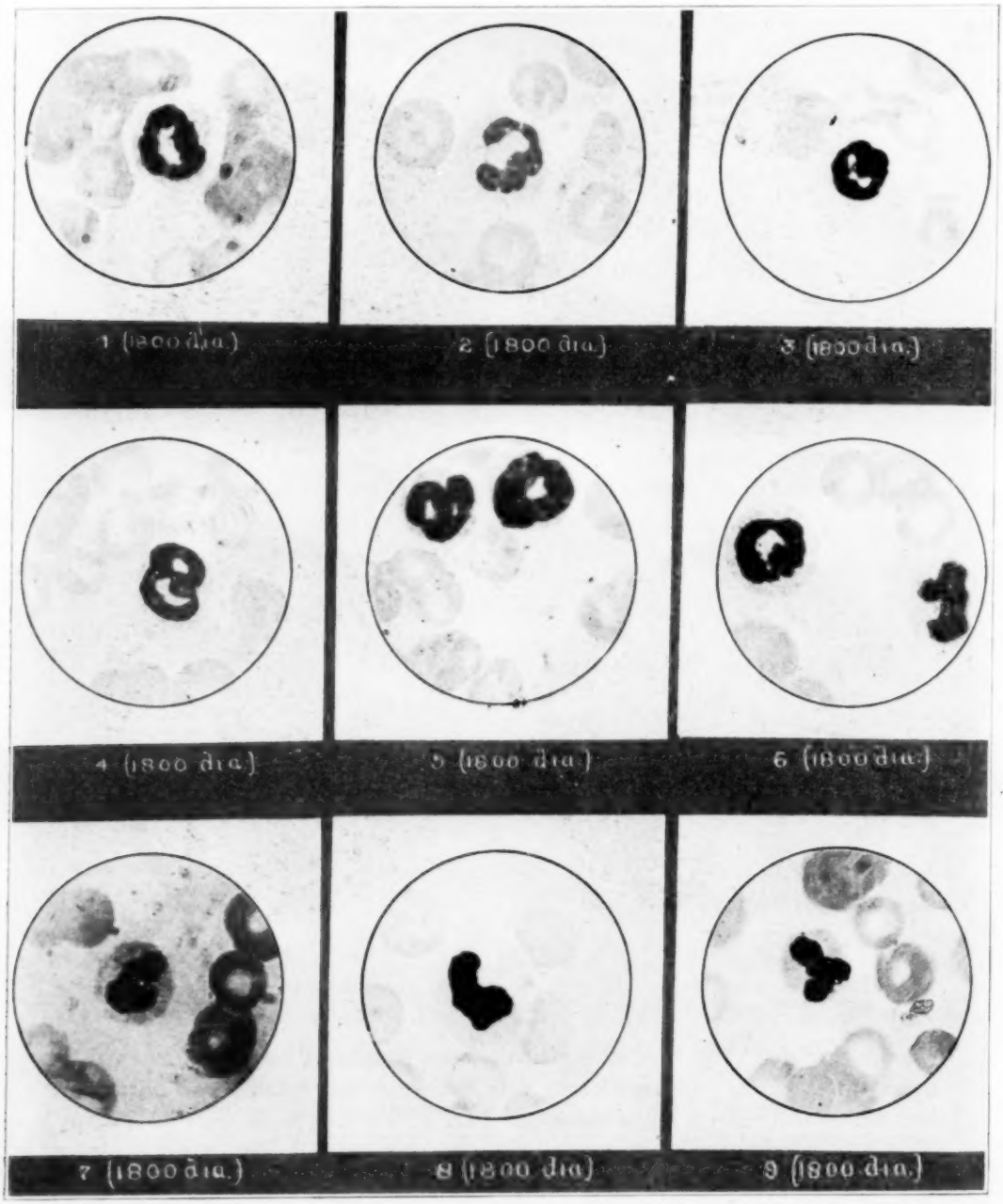

Fig. 6.—Atypical neutrophils in the writer's blood during oatmeal diet. (October 31, 1916.) Ringforms, 6 per cent: Bizarre forms 5 per cent. No. 5, large lymphocyte to right.

showing a strong resemblance to a figure eight may be found in Fig. 4, No. 4 and Fig. 7, No. 3. Whatever the cause of the phenomenon may be there seems to be a curious tendency to pseudo-ring formation both in the blood of the carcinoma patient and in that of the writer when the blood cholesterol reaches its maximum. The gradual closing of the ring in the blood of a carcinoma

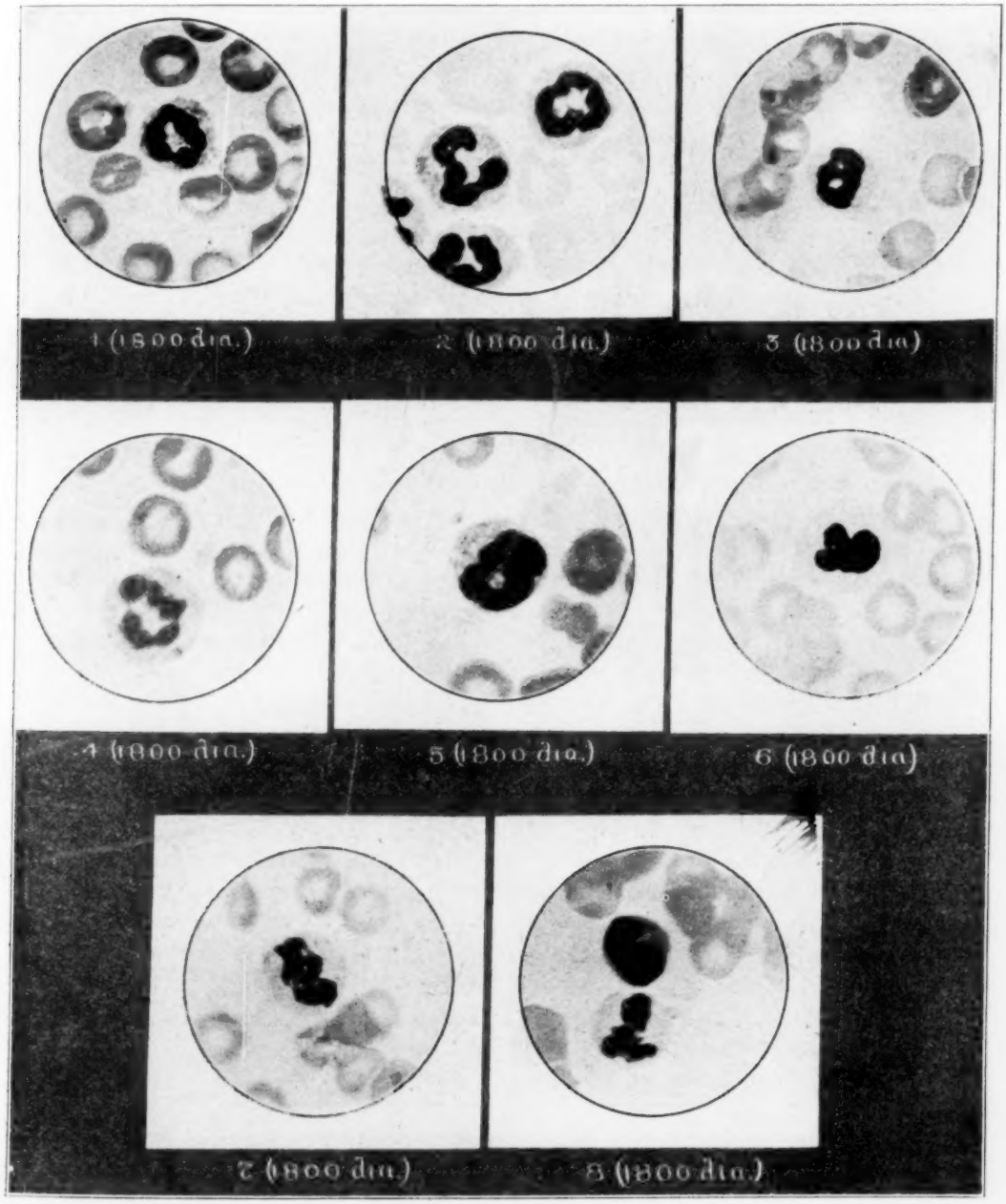

Fig. 7.—Atypical neutrophils in the writer's blood during oatmeal diet. (November 1, 1916.) Ringforms, 6 per cent: Bizarre, 7 per cent. No. 5, large lymphocyte.

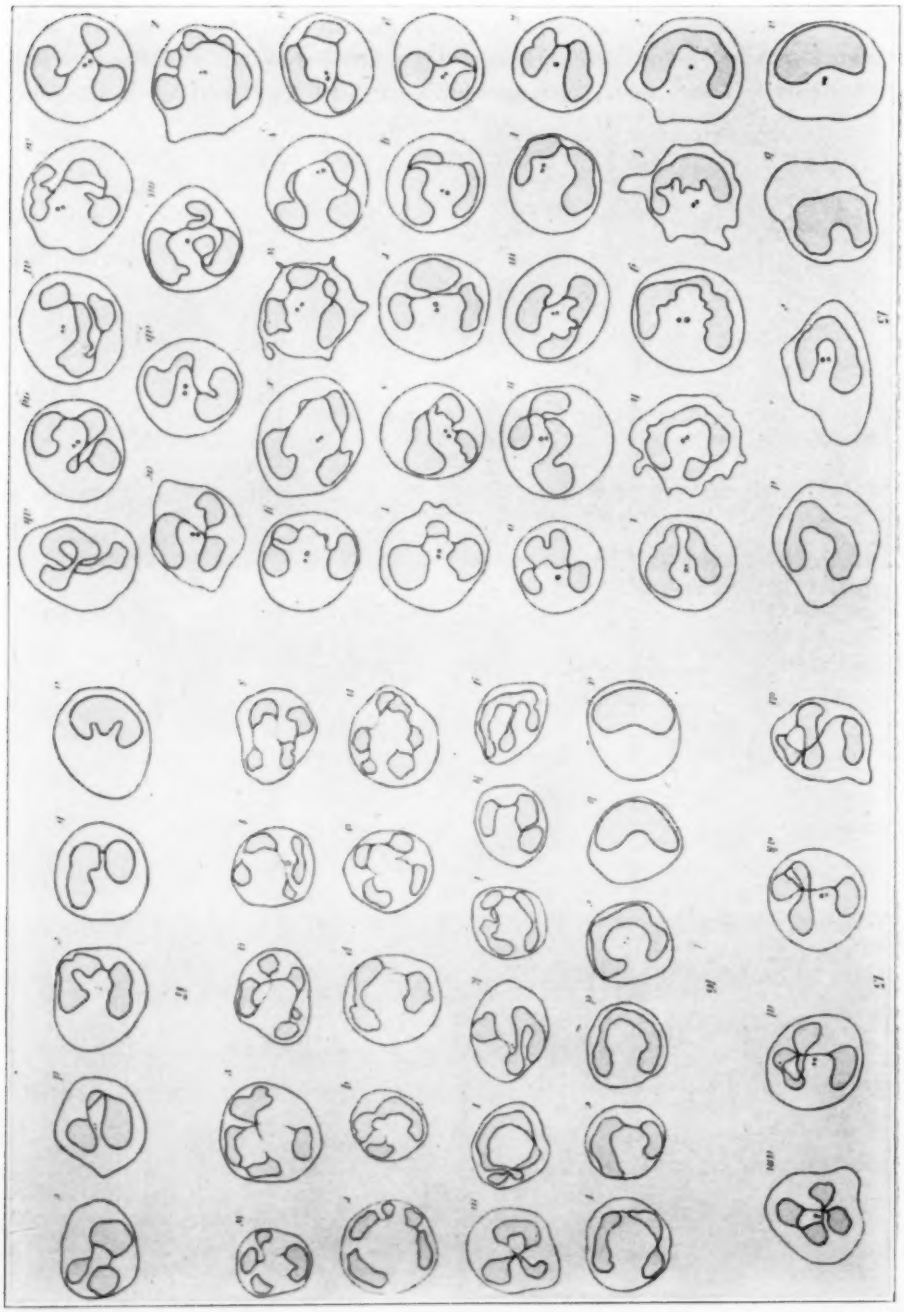

Fig. 8.—Evolution of the nucleus in the neutrophil polymorphonuclear leucocytes. (After Weidenreich.) No. 15, *a* to *am*: Normal human blood. No. 16, *a* to *w*: Leucemic human blood. No. 17, *a* to *e*: Eosinophile leucocytes in leucemic blood. Observe that the only nucleus of the ring type resembling the writer's photomicrographs is in the leucemic blood: No. 16, 1.

patient may be traced in Fig. 1, No. 3. In the blood of the writer, ring-like forms were found even among the large lymphocytes (Fig. 4, No. 4; Fig. 6, No. 5; Fig. 7, No. 5). The morphologic evolution of the nucleus, described by Weidenreich, is illustrated by a reproduction of one of his illustrations (Fig. 8). The fact that the only cell in the diagrams which bears a close resemblance to the writer's photomicrographs, was found by Weidenreich in leukemic blood (Fig. 8, No. 16, 1) may be more than a mere coincidence, since the relation of leucemia, pernicious anemia and carcinoma has been discussed by hematologists as well as pathologists (Pappenheim, Heinrichsdorff, Slye,[50] and Slye, Holmes and Wells[49]). "The leukemic process is to be regarded as a generalized proliferation (Der leukamische Prozess ist als eine generalisierte Wucherung aufzufassend" Grawitz, page 244.) (Figs. 1, 2, 3, 4, 6, 7, and 8.)

Definite conclusions concerning the diagnostic value of these atypical neutrophils are undoubtedly premature, but the fact that their numerical increase is directly proportional to the cholesterol value; i. e., the chemical composition of the blood, both in the diet experiments here reported and in an advanced case of carcinoma, and inversely proportional to the lymphoid count, may be significant; it recalls Weidenreich's suggestion that the study of the atypical leucocytes might not be without practical value and "might open up new perspectives" ("es ware nicht unmoglich dass sich dadurch neue Gesichtspunkte gewinnen liessen").

That the chemical composition of the blood can be changed by dietetic measures within a few days has been shown repeatedly in these investigations. The blood cholesterol was reduced by one-third in two days by means of Gruner's diet and increased as much in three to four days by the meat and oatmeal experiments. Now if the cytology of the blood and even the morphology of the neutrophil leucocytes can be influenced by the chemical composition of the blood to the extent seen in the experiments here reported, it is hardly to be expected that other cells should be insensitive to this influence. We know that the life of every cell in the body depends on its blood supply. We have seen that the chemical composition of the blood depends to a certain extent, at least, on the chemical composition of the diet. If we consider that dietary conditions may continue unaltered for months and years and that the chemical composition of the blood will affect body cells during this entire period, the influence of the diet on cell growth or cell proliferation can not be discarded as merely hypothetical.

There is every reason to assume that cholesterol plays a part in the formation of new cells. If we admit that cancer, i. e., undue proliferation, almost invariably occurs on the site of old lesions where the normal process of reparative proliferation is already active and further stimulation will be most readily responded to, the significance of the cholesterol content of the blood can not be ignored.

The inheritability of the tendency to tumor formation has been proved repeatedly by Slye[49, 50, 51, 52, 53] in her observations on mice, and the inheritability of specific organs has been shown conclusively by her brilliant report on tumors of the liver[52] in the same animals. In this report sixty-two primary

liver tumors could be traced in the direct descendants of one female mouse with a malignant adenoma of the liver and a sarcoma of the mammary gland.*

The number of liver tumors and their spontaneous transmission in Slye's mice are equally remarkable as only one tumor of the liver in mice has been reported elsewhere. From the inheritability of specially disposed organs the hereditary transmission of inadequate organs may be logically deduced and if the organs thus transmitted should be among those that take a prominent part in metabolic function (the liver, for instance), faulty metabolism can reasonably be expected in the generations that inherit such organs. The tendency shown by certain types of cancer to appear in various members of human families is not unknown in medical circles,[22, 38, 60] and the assumption that the influence of metabolism on this tendency may be important is supported by Burrow's conclusions from his experiments on the growth of tissue *in vitro:* "If these experiments are substantiated . . . . . the problem of cell growth is brought into the domain of chemistry. Thus problems, such as confront us in cancer, are greatly narrowed." It is the writer's conviction that faulty metabolism; i. e., the inability of the organism to metabolize certain kinds of food (meat may be among these), may play a far greater part in the incidence of cancer than has, hitherto, been generally accepted.

CONCLUSIONS

A consideration of the work of other observers and of the writer's experiments herein detailed seems to warrant the following conclusions:

1. The influence of the chemical composition of the food on the chemical composition of the blood in increasing or diminishing the amount of cholesterol therein is clearly demonstrated.

2. A diet which increases the blood cholesterol coincidently weakens the lymphoid defense.

3. A diet which reduces the blood cholesterol coincidently increases the lymphoid defense.

4. In persons predisposed to carcinoma an increase of the cholesterol and a weakening of the lymphoid defense such as may occur with the prolonged use of a diet adapted thereto, may perhaps result in the development of carcinoma.

5. Dietetic measures calculated to reduce the blood cholesterol and coincidently increase the lymphoid defense may yet prove to be of value in the treatment of carcinoma.

BIBLIOGRAPHY

[1]Aschoff, L.: Cited by Rothschild,[44] p. 230: Personal communication to Rothschild concerning the presence of gall stones after antifat cures (Entfettungskuren).

[2]Autenrieth. W., and Funk, A.: Ueber kolorimetrische Bestimmungsmethoden: Die Be timmung des Gesamtcholesterins im Blut und in Organen, München. med. Wchnschr., 1913, i, 1243-1248.

[3]Bloor, W. R.: Studies on Blood Fat, II. Fat Absorption and the Blood Lipoids, Jour. Biol. Chem., 1915. xxiii, 317-326.

[4]Bloor. W. R.: The Determination of Cholesterol in Blood, Jour. Biol. Chem., 1916, xxiv, 227-231.

*Slye's mice are used for breeding purposes only and the tumors found among them are spontaneous growths in every instance. The incidence of spontaneous tumors in rats has been studied lately by Bullock and Rohdenburg.

[5]Bloor, W. R., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, Jour. Biol. Chem., 1916, xxvii, 107-112.

[6]Bloor, W. R.: The Distribution of Lipoids ("Fat") in Human Blood, Jour. Biol. Chem., 1916, xxv, 577-599.

[7]Browder, A.: The Effect of Lecithin and Cholesterol Upon the Division Rate of Paramecium, Univ. California Pub. in Physiol., 1915, v, 1-3.

[8]Bulkley, L. D.: The Relation of Diet to Cancer, Med. Rec., New York, 1914, lxxxvi, 699-702.

[9]Bullock, F. D., and Rohdenburg, G. L.: Spontaneous Tumors of the Rat, Jour. Cancer Research, 1917, ii, 39-60.

[10]Burrows, M. T.: Some Factors Regulating Growth, Proc. Am. Assn. Anatomists, 1916, Abst. in Anat. Rec., 1917, xi, 335-339.

[11]Chalatow, S. S.: Über das Verhalten der Leber gegenüber den verschiedenen Arten von Speisefett, Virchows Arch. f. path. Anat., 1912, ccvii, 452-476.

[12]Dezani, S.: Ricerche sulla genesi della cholesterina, I, Gior. d. r. Accad. di med. di Torino, 1913, 4s., xix, 149-158.

[13]Dezani, S.: Richerche sulla genesi della cholesterina, II, Arch. farmacol. speriment., 1914, xii, 4-12.

[14]Downey, H.: Personal communication.

[15]Fodor, A.: Sterine. In Abderhalden, E.: Biochemisches Handlexikon, Springer, Berlin, 1914, viii, 474, 476.

[16]Foote, N. C.: Ueber das Verhalten des Hühnerknochenmarks gegen Immunplasma in den Zellkulturen nach Carrel, Centralbl. f. allg. Path. u. path. Anat., 1912, xxiii, 577-581.

[17]Foote, N. C.: Über das Wachstum von Knochenmark in vitro, Experimenteller Beiträge zur Entstehung des Fettgewebes, Beitr. z. path. Anat. u. z. allg. Path., 1912, liii, 446-476.

[18]Friedenwald, J., and Ruhräh, J.: Diet in Health and Disease, W. B. Saunders Co., 1913.

[19]Gardner, J. A., and Lander, P. E.: The Origin and Destiny of Cholesterol in the Animal Organism. Part XI. The Cholesterol Content of Growing Chickens under Different Diets, Proc. Roy. Med. and Chir. Soc., London, Series B, 1913-14, lxxxvii, 229-236, cited by Bloor,[6] page 582.

[20]Grawitz, E.: Klinische Pathologie des Blutes, Leipzig, 1911, Thieme, 204.

[21]Gruner, O. C.: A Study of the Changes Met with in the Leucocytes in Certain Cases of Malignant Disease, Brit. Jour. Surg., 1916, iii, 506-525.

[22]Hedinger, E.: Primärer Leberkrebs bei zwei Schwestern, Centralbl. f. allg. Path. u. path. Anat., 1915, xxvi, 385-387.

[23]Heinrichsdorff, A.: Ueber die Beziehungen der perniziösen Anämie zum Karzinom, Folia haemat., 1912, xiv, Abst. in Centralbl. f. allg. Path. u. path. Anat., 1915, xxvi, 113.

[24]Hoffman, F. L.: The Mortality from Cancer in the Western Hemisphere, Jour. Cancer Research, 1916, i, 21-48.

[25]Joest, E., and Ernesti, S.: Untersuchungen über spontane Geschwülste, bei Vögeln mit besonderer Berücksichtigung des Haushuhns, Ztschr. f. Krebsforsch., 1915, xv, 1-75.

[26]Kendall, E. C.: The Isolation in Crystalline Form of the Compound Containing Iodin which Occurs in the Thyroid; Its Chemical Nature and Physiological Activity, Tr. Assn. Am. Phys., 1915.

[27]Krylow, D. D.: Experimentelle Studien über Nebennierenrinde, Beitr. z. path. Anat. u. z. allg. Path., 1914, lviii, 434-467.

[28]Luden, G.: Observations on Cholesterol Retention as a Factor in Cell Proliferation, Jour. Lab. and Clin. Research, 1916, ix, 662-676.

[29]Luden, G.: Observations on the Changes in the Cholesterol Content of the Blood of Goats Following Cholesterol Feeding Alone, Roentgen Treatment Alone, and Cholesterol Feeding Combined with Roentgen Treatment and Subsequent Castration, Jour. Biol. Chem., 1916, xxvii, 273-295.

[30]Levy, R. L., and Rowntree, L. G.: A Study of the Buffer Value of the Blood, Arch. Int. Med., 1916, xvii, 525-539.

[31]McMeans, J. W.: Tissue Reactions in Experimental Hypercholesterinemia, Jour. Med. Research, 1916, xxxiii, 481-491.

[32]McNee, J. W.: Cholesterin: An Account of Its Relations to Pathology and Physiology, Quart. Jour. Med., 1914, vii, 221-236.

[33]Müller, F., von, and Bauer, J., von: In their clinical lectures attended by the writer. (Post-Graduate Courses in Clinical Medicine, Munich, 1910-1912.)

[34]Mueller, J. H.: The Mechanism of Cholesterol Absorption, Jour. Biol. Chem., 1916, xxvii, 463-480.

[35]Mueller, J. H.: A Comparison of the Results Obtained by the Colorimetric and Gravimetric Determinations of Cholesterol, Jour. Biol. Chem., 1916, xxv, 549-560.

[36]Murphy, J. B., and Morton, J. J.: The Lymphocyte in Natural and Induced Resistance to Transplanted Cancer, II. Studies in Lymphoid Activity, Jour. Exper. Med., 1915, xxii, 204-211.

[37]Pappenheim, A.: Perniziöse Anämie und Karzinom in ihren gegenseitigen Beziehungen, Folia haemat., 1912-13, xiv, 329-358.

[38]Pel, P. K.: Familien-Magenkrebs, Berl. klin. Wchnschr., 1915, lii, 288-289.

[39]Robertson, T. B., and Burnett, T. C.: The Influence of Lecithin and Cholesterin upon the Growth of Tumors, Jour. Exper. Med., 1913, xvii, 344-352.

[40]Robertson, T. B.: Experimental Studies on Growth. VIII. The Influence of a Diet Deficient in Fats, and of the Same Diet with Cholesterol Added, upon the Growth of the White Mouse, Jour. Biol. Chem., 1916, xxvii, 393-402.

[41]Rothschild, M. A.: The Relation of the Liver to the Cholesterin Metabolism, Proc. New York Path. Soc., 1914, n.s., xiv, 229-236.

[42]Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. III. Die Beziehungen der Nebenniere zum Cholesterinstoffwechsel, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 39-65.

[43]Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. IV. Über die Beziehungen der Leber zum Cholesterinstoffwechsel, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 1914, lx, 66-75.

[44]Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. V. Der Cholesteringehalt des Blutes und einiger Organe im Hungerstand, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 227-231.

[45]Rothschild, M. A., and Rosenthal, N.: The Dietetic Management of Hypercholesterinemia in Cases of Cholelithiasis, Am. Jour. Med. Sc., 1916, clii, 394-403.

[46]Rowntree, L. G.: Personal communication.

[47]Saltykow, S.: Experimentelle Atherosklerose, Beitr. z. path. Anat. u. z. allg. Path., 1914, lvii, 415-473.

[48]Simon, C. E.: Personal communication.

[49]Slye, M., Holmes, H. F., and Wells, H. G.: The Primary Spontaneous Tumors of the Lungs in Mice, Studies on the Incidence and Inheritability of Spontaneous Tumors in Mice; Fourth communication, Jour. Med. Research, 1914, xxx, 417-442.

[50]Slye, M.: The Incidence and Inheritability of Spontaneous Cancer in Mice; Third report, Jour. Med. Research, 1915, xxxii, 159-200.

[51]Slye, M.: The Inheritability of Spontaneous Tumors of Specific Organs and of Specific Types in Mice. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice; Fifth report, Jour. Cancer Research, 1916, i, 479-502.

[52]Slye, M.: The Inheritability of Spontaneous Tumors of the Liver in Mice. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice; Seventh report, Jour. Cancer Research, 1916, i, 503-522.

[53]Slye, M., Holmes, H. F., and Wells, H. G.: Primary Spontaneous Sarcoma in Mice; Eighth communication, Jour. Cancer Research, 1917, ii, 1-38.

[54]Soper, W. B.: Zur Physiologie des Cholesterinstoffwechsels. VI. Ueber Beziehungen der Milz zum Cholesterinstoffwechsel, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 232-244.

[55]Stahr, H.: Durch andauernde Haferfütterung erzeugtes Epitheliom der Rattenzunge, Beitr. z. path. Anat. u. z. allg. Path., 1915, lxi, 169-235.

[56]Sternberg, H.: Die Nebenniere bei physiologischer (Schwangerschaft-) und artifizieller Hypercholesterinämie, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 91-123.

[57]Stevens, R. H.: Blood in Cancer under Roentgen Therapy, Proc. Am. Roentgen Ray Soc., 1916, Abst. in Jour. Am. Med. Assn., 1916, lxvii, 1549.

[58]Weidenreich, F.: Beiträge zur Kenntnis der granulierten Leucocyten. V. Fortsetzung der Studien über das Blut und die blutbildenden und zerstörenden organe., Arch. f. mikros. Anat., 1908, lxxii, 209-325.

[59]Williams, W. R.: The Natural History of Cancer, with Special Reference to its Causation and Prevention, London, 1908, Heineman, 214.

[60]Wolff, J.: Die Lehre von der Krebskrankheit von den ältesten Zeiten bis zur Gegenwart, Fischer, Jena, 1914, 663. Cited by Hedinger.[22]

[61]Ziegler, E.: Cited by Naegeli, O.: Blutkrankheiten und Blutdiagnostik; Lehrbuch der morphologische Hämatologie, 2 ed., Leipzig, 1912, Veit, 181.

ON THE DEVELOPMENT OF A METHOD FOR EARLY DIAGNOSIS OF TUBERCULOSIS BY THE USE OF THE X-RAYED GUINEA PIG*

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THE inoculation method has long been recognized and still stands as the most reliable test at our command for the diagnosis of early or obscure tuberculous lesions. The chief objections to the method are that a positive virus requires a long time to develop in the inoculated animal and that some animals have sufficient natural resistance to overcome a mild infection. For these reasons any means of effectively breaking an animal's resistance so that the length of time is decreased and the "take" made more certain is worthy of a thorough trial.

Murphy and Ellis[1] showed that white mice were remarkably more susceptible to bovine tuberculosis after having been exposed to the x-ray than were normal animals. They offered as an explanation the destruction of the lymphoid tissue which they cited as an important factor in the defensive mechanism against tuberculosis. Morton,[2] assuming that the guinea pig would be affected in a like manner, tried the x-ray on a series of inoculations. He reported that when animals were subjected to a massive dose of x-ray about the time of inoculation, they were so sensitized as to reduce the time of development of recognizable lesions to ten days. He intimated that repeated exposures might be more effective but were unnecessary. He found marked reductions in the leucocyte counts following the exposure.

Haythorn and Lacy,[3] while carrying out some studies in the healing process of tuberculous lesions treated by the x-ray, noted the continued fall in the resistance and the rapid extension of the lesions under frequently repeated large doses of x-ray. Their findings confirmed the decrease in the total number of leucocytes after a large dose of x-ray, but they did not find the decrease in the lymphocytes as described by Murphy and Ellis. They suggested that the well known inhibiting influence of x-ray on the glands of internal secretion might be in a measure responsible for the decreased resistance of the animal.

With the idea of working out a practical routine procedure for sensitizing guinea pigs in the rapid diagnosis of tuberculosis by means of the inoculation method a small group of animals weighing as nearly 260 grams each, as it was possible to get, were inoculated with a uniform dose of tubercle bacilli and given different doses of x-ray at varying intervals. The uniform dose of tubercle bacilli was obtained by adding a few loops of tubercle bacilli from a culture of human bacillus tuberculosis to normal salt and placing the mixture in a vaccine bottle containing glass beads and shaking them for one hour in an electric shaker. An equal amount of 20 per cent India ink† in normal salt was added and one-half cubic centimeter of the suspension injected into both the groin and the peritoneal

*Reported from the William H. Singer Memorial Research Laboratory, Pittsburgh, Pa.

†The India ink was introduced for the purpose of marking the tubercles so that the results could be used in connection with a separate piece of work.

sac of each of the animals. As the whole group was inoculated from the same suspension, each animal received approximately the same dose of the tubercle bacilli. The dose of x-ray was given with a Coolidge tube. The current was 60,000 volts, spark gap six inches; filter one half millimeter of aluminum; target twelve inches; length of time five minutes. The animals were examined each day and the effects on general condition, time of appearance of nodules, progress of lesions, etc., were noted. Leucocyte and differential counts were made regularly.

Two inoculated animals and a control which received pigment only, were given a single massive dose lasting ten minutes. This was twice the dose given the other animals. All three developed nodules on the fourth day. By the eighth day the nodule in the control had disappeared and those in the inoculated animals had enlarged to the size of a cherry stone. One remained about the same in appearance, the other developed additional nodules, which later united to form a mass one and one-half centimeters in diameter. All three animals retained their sleekness and showed little emaciation at the end of five weeks. They all continued to grow. Leucocyte counts were made on the day before, the day after, and every fifth day after the x-ray exposure. The results were unconvincing. There was an initial leucocytosis due probably to the injection of pigment. This was followed by a decrease to about 50 per cent of the normal count. The differential counts showed the first rise to be due to an increase in polymorphonuclear leucocytes. The later differential counts showed a return to the same relative percentages that were found in the original counts.

A second set of two animals and a control were given the usual dose, the control receiving pigment alone, and were treated on alternate days with a five minute dose of x-ray. The two which received the tubercle bacilli showed nodules in four days, which continued to enlarge. The nodules in one broke down and healed over twice and the animal died on the thirty-third day. At autopsy this animal was found to be greatly emaciated and showed miliary tuberculosis of the peritoneum, lymph nodes, liver, spleen, and lungs. The other animal ran a similar but somewhat longer course. The control showed a nodule at first which had cleared up entirely by the eighth day. The general condition of these animals was bad at all times, they did not grow and while the lesions did not appear any earlier, the extension and development appeared to be more rapid. Leucocyte and differential counts were made on the day following each dose of x-ray. All three animals showed an initial polymorphonuclear leucocytosis followed by a gradual drop to an average of 53 per cent of the original count. The relative number of leucocytes and lymphocytes remained practically the same.

A third set of three was treated on every sixth day and one animal was treated on every third, but the results were so similar to those just cited that no new points can be brought out by giving them in detail. Two animals which received no injections were given x-ray treatments on alternate days, and the leucocytes counted. They showed no initial rise, but the decrease in the cells were exactly similar to those found in the ones which received the inoculations. There was no relative decrease in the lymphocytes. The controls which received neither inoculations nor treatments remained healthy and grew rapidly.

The Singer Laboratory has, since the use of the x-ray for the purpose of

sensitizing animals was begun, the records of fifty-six animals that have been inoculated either with known tuberculous or suspected tuberculous material. In several instances material from a patient has been injected into two animals at the same time, one animal being subjected to x-ray while the other was not. In one such instance both animals showed lesions on the same day, but the rayed nodule outstripped the other in the rapidity of development. In another instance the x-ray was not used until several days after the inoculation, and no nodules had appeared at the time its use was begun. Doses were then given on alternate days and after the second exposure a nodule appeared and developed rapidly.

One animal which had been injected with an unknown fluid for diagnosis was killed and autopsied as soon as a nodule appeared, but neither bacteriologic nor microscopic examination showed the presence of tuberculosis. It later developed that the patient from whom the fluid had been taken had tuberculosis, but the test failed because the animal was killed before the lesions were sufficiently developed for diagnosis. In several other instances growing nodules were removed under local anesthesia and examined, the animal being allowed to live so that the material injected into the peritoneum, if positve, would continue to develop, even if the gland findings were negative. The earliest nodule removed was on the fourth day after inoculation, but neither the bacilli nor characteristic changes were found. One animal, which received a very large dose of known tuberculous material, was autopsied on the tenth day, and tubercle bacilli were present in the smears from the abdominal organs. The glands from the groin showed the earliest recognizable histologic tuberculous rosettes. Most of the animals which showed positive signs were not autopsied under two weeks.

Throughout the work it was obvious that the rapidity of appearance and development of the nodules depended more on the size of the dose of tubercle bacilli than upon the amount of x-ray given.

Based largely upon the above findings the following routine method for using x-rayed guinea pigs in the early diagnosis of tuberculosis, is recommended.

The material must first be prepared for injection. If it is sputum or is of a mucoid nature, it is subjected to fifteen minutes digestion with 4 per cent potassium hydroxide after Petroff's method and is centrifuged and washed before injection. If it is of a fibrinous nature, it is digested in 15 per cent antiformin, the length of time depending upon the resistance of the material to digestion. If it is a clear fluid, it is merely centrifuged and the sediment taken. From one-half to one cubic centimeter of the prepared material is injected into both the groin and the abdomen of the animal, which is then exposed every second day to a five-minute dose of x-ray for at least three exposures. If a nodule has appeared and is still present in two weeks, it is removed under a local anesthetic, smeared, and examined histologically. If positive, it is reported at once; if negative, the animal should be kept for at least six weeks so that in case a few bacilli are present in the cavity they may develop independently from the node in the groin. If the animal is negative in a thoroughly "rayed pig" at the end of six weeks the test may safely be called "negative."

Even in this very small series several points stand out prominently.

1. It is possible by means of the x-ray to increase the susceptibility of guinea

pigs to tuberculosis. Frequently-repeated doses have a more certain effect than a single dose.

2. The destruction of lymphoid cells in itself is insufficient to explain the decreased resistance.

3. The removal of a nodule from the groin for examination can be done in about two weeks and is usually diagnostic. This procedure has the advantage that in case of negative local findings the animal has the chance of developing internal lesions at a slightly later time.

I wish to express my appreciation to Doctors Haythorn, Lacy, and Allison for their interest and suggestions during this work.

BIBLIOGRAPHY

[1]Murphy, Jas. B., and Ellis, A. W. M.: Jour. Exper. Med., 1914, xx, 397.

[2]Morton, J. J.: Jour. Exper. Med., 1916, xxiv, 419.

[3]Haythorn, S. R., and Lacy, G. R.: The Effect of X-Ray on Experimental Tubercles Marked with Pigment. Read before the American Association of Pathologists and Bacteriologists, April 7, 1917.

TUNGSTATES OF ALKALOIDS—AN EXPERIMENTAL PHARMACOLOGIC STUDY*

By Bernard Fantus, M.S., M.D., Chicago, Ill.

INTRODUCTION

THOUGH tungstates have been used as reagents for alkaloids for many years, the only study of the pharmacologic effects of these alkaloidal combinations, I have been able to find, has been by Carl Nielson,[1] who showed that emetine phosphotungstate is practically nonemetic in dogs, that strychnine phosphotungstate produces convulsions in frogs and in dogs, it being merely somewhat less toxic and slower in action than strychnine sulphate, and that quinine phosphotungstate given to a puppy in doses of 4.65 grains three times daily for ten days, corresponding to a total administration of 117 grains of phosphotungstic acid, produced no evident effects. Inasmuch as the phosphotungstates are much less bitter than the readily soluble salts of the alkaloids, and apparently have less effect upon the stomach, it seemed a subject worthy of further study with a view of the possibility of using these alkaloidal precipitates as an administration form for alkaloids.

There are two questions that must be answered before alkaloidal double tungstates could be seriously recommended for practical use in therapeutics: first, the degree of toxicity or of harmlessness of tungsten and the tungsten acids; and, second, the degree to which the action of the various alkaloids is modified by combination with double tungstates.

Metallic tungsten is considered so harmless that it has been advocated by Richard Krüger[2] as a substitute for bismuth salts in roentgenography of the stomach and intestine. Krüger gave 25 to 80 gm. of colloidal tungsten to human beings without ill effects of any kind.

The most extensive study of the pharmacology of tungsten, at present available, is that of Jacob Bernstein-Kohan,[3] who found that a solution containing less than 5 per cent of sodium tungstate did not produce toxic effects on paramecia, worms or other low organisms. The smallest lethal dose of tungsten, calculated as metal, on subcutaneous administration of sodium tungstate, Bernstein-Kohan found to be for various animal species as follows (Table I):

Table I

Minimum Lethal Dose per Kg. of Animal on Subcutaneous Administration of Sodium Tungstate, Calculated as Metal. (Bernstein-Kohan.)		
Frog:	About 0.450 gm.	Lived 12 to 13 hours.
Rat:	0.338 "	" 6 to 10 "
Rabbit:	0.044 "	" 2 to 3 "
Cat:	0.111 "	" 12 to 14 "
Dog:	0.078 "	
Rooster:	0.250 "	" 20 to 22 "

*This research was commenced at the Laboratory of Pharmacology of the University of Michigan and completed at the Pharmacologic Laboratory of the University of Illinois.

In marked contradistinction to the toxicity of sodium tungstate on hypodermic administration, Berstein-Kohan obtained negative results on oral administration to rabbits of 0.017 gm. daily for 45 days. He concluded that it is not absorbed or only very sparingly absorbed through the gastrointestinal mucosa.

Kobert's[4] statements regarding our knowledge on tungsten may be summarized as follows: The water soluble salts of the metal, especially tungstic acid, as well as phosphotungstic acid, are as caustic as the soluble iron salts or even as chromic acid. They produce vomiting by local irritation. Noncorrosive tungsten compounds are, like the corresponding iron compounds, absorbed with difficulty from the intestine. But little is known about their toxic action, when they are given by mouth. When introduced subcutaneously into the body, they are deposited, like iron, in the liver, spleen, muscles, kidney, skin, and especially the bones. Elimination occurs chiefly through the mucosa of the alimentary tract with liability to production of gastroenteritis, especially the diphtheritic form of dysentery. It is eliminated to a lesser degree in the urine. Still tungsten may produce a mild degree of nephritis and hemorrhages from the kidney.

Kobert notes that the use of tungsten-containing dyes is prohibited in Berlin on Police Order of Oct. 25, 1878, though neither corrosion nor other damage has been reported from them.

Kunkel[5] also notes that economic, industrial, or experimental intoxication from oral administration of tungsten has not been reported; and that, owing to slight absorption from the intestinal tract, its occurrence is not at all probable.

PRESENT STUDY

This work was carried on with phosphotungstic and silicotungstic acids. The latter was used chiefly, because it was found that most of the alkaloidal silicotungstates were less bitter than the phosphotungstates.

Inasmuch as silicotungstic acid does not seem to be readily obtainable on the market, it might be well to describe here the process I employed in the preparation of the substance used in these experiments.

Preparation of Potassium Silicotungstate.[6]—To 40 gm. Na_2SiO_4 dissolved in 200 ml. water, to make a fairly saturated solution, add 20 ml. conc. HNO_3 or until the fluid produces a slight reddening of litmus paper. Filter.

To the SiO_2, thus produced, was added a solution of 120 gm. Na_2WO_3 in 300 ml. of water (to make the total weight of fluid about 8 times that of WO_3 used). The mixture was next strongly acidified with (75 ml.) $HC_2H_3O_2$ and heated to boiling.

When the fluid is no longer precipitated within a few moments by HCl, permit to cool, filter the feebly acid solution, and add ether and liberal amounts (800 ml. or more) of 33 per cent H_2SO_4 until a turbidity is produced on stirring, and three strata are formed on standing. The lower stratum, a molecular combination of ether with silicotungstic acid is, after clearing, separated and decomposed by water. The ether is driven off, and the potassium salts are formed in the following manner:

Into a weighed crucible, place a definite amount, say 2 ml., of the liquid, evaporate to dryness, and then heat to redness. Weigh the residue (which is

chiefly SiO_2, 12 WO_3), and add to 2844 parts of the latter a watery solution of 276 parts of K_2CO_3, and concentrate on the water-bath.

By fractional crystallization, two compounds may be separated from each other. At first crystallizes 2 K_2O, SiO_2, 12 WO_3, 18 H_2O, in fine hexagonal prisms, which are filtered off as soon as the more soluble orthorhombic crystals of 2 K_2O, SiO_2, 13 WO_3, 9 H_2O commence to appear. The yield is about 50 per cent of the Na_2 WO_4 used.

1. *Experiments on the Toxicity of Double Tungstates*

Hypodermic Administration.—That both phosphotungstic and silicotungstic acids are toxic on subcutaneous administration to guinea pigs, will be seen from Table II which shows the results of injections of 10 per cent solutions of the respective acids.

TABLE II

COMPARATIVE TOXICITY OF PHOSPHOTUNGSTIC AND SILICOTUNGSTIC ACIDS ON GUINEA PIGS

	PHOSPHOTUNGSTIC ACID		SILICOTUNGSTIC ACID	
DOSE MG. PER GM.	NO. OF EXPERIMENTS	RESULTS	NO. OF EXPERIMENTS	RESULTS
0.25	2	Recovered	2	Recovered
0.50	4	All died	6	All died
1.00	1	Died	1	Died

It will be seen that the two acids have the same degree of toxicity, at least within the rather wide limits of this experiment. A dose of ½ mg. per gram or 0.5 gm. per kg. is uniformly fatal; ¼ mg. per gm. is recovered from. After a dose of 0.5 gm. per kg., the symptoms do not appear for an hour or two. Then the animal develops depression, so that it remains on the side or on the back, when placed in these positions; the breathing becomes labored; and death occurs in from 1 to 3 days. Necropsy shows local irritation and even necrosis at the point of injection.

Oral Administration.—In strong contrast to the toxicity of the tungstates on subcutaneous injection is their comparative harmlessness when administered by mouth, as will be seen from the protocols of the following experiments:

PROTOCOL OF PUPPY 1

DATE	DOSE BY STOMACH TUBE	WEIGHT
Aug. 3	0	1.830 gm.
" 8	0.5 gm. Potass. Silicotungstate	1,980 "
" 9	0.5 " " "	2,050 "
" 10	0.5 " " "	1,920 "
" 11	0.5 " " "	1,955 "
" 12	0.5 " " "	1,850 "
" 13	0	
" 14	0.5 gm. Potass. Silicotungstate	2,120 "
" 15	0.5 " " "	2,000 "
" 16	0.5 " " "	2,010 "
" 17	0.5 " " "	2,080 "
" 18	0	2,120 "
" 19	0	2,130 "

Animal seems normal. Urine free from albumin.

The moderate loss of weight produced in Puppy 1, which consumed 4.5 gm. of potassium silicotungstate in nine days, was probably due to temporary digestive impairment, for it was more than made up during the day's interval, on which the drug was not given.

PROTOCOL OF RABBIT 1

DATE	DAILY FOOD WITH DRUG		(GIVEN DAILY)	URINE QUANT. ML.	WEIGHT (GM.)	REMARKS
	OATS	CARROTS				
Oct. 25	20	100	No medicine	No	925	Ate all
26	"	"	" "	"	930	" "
27	"	"	" "	33	945	" "
28 & 29	40	200	" "	38	945	" "
30	20	100	" "	30	930	" "
31	"	"	" "	21	960	" "
Nov. 1	"	"	" "	20	950	" "
2	"	"	+0.20 Silico-tungstic Acid	10	960	" "
3	"	"	"		970	" "
4 & 5	40	200	+0.40 Silico-tungstic Acid	48	975	" "
6	20	100	+0.20 Silico-tungstic Acid	63	965	" "
7	"	"	"	No		" "
8	"	"	"			" "
9	"	"	"	26	910	" "
10	"	"	"	5	885	Left 30 gm. food
11 & 12	40	200	+0.40 Silico-tung. Acid	38	865	" 65 " "
13	20	100	+0.20 Silico-tung. Acid	45	800	" 60 " "
14	"	"	+0.10 Silico-tung. Acid	23	775	" 30 " "
15	"	"	"	19	770	" 30 " "
16	"	"	"	15	760	" 40 " "
17				5	Died	" 57 " "

The rabbit consumed, in the course of 15 days, 2.3 gm. of silicotungstic acid mixed with its food before it died. Incomplete necropsy showed a small hemorrhage in the cecum and blood stain around the anus.

PROTOCOL OF CAT 1 (BLACK AND BROWN)

DATE	DAILY FOOD WITH DRUG		(GIVEN DAILY)	WEIGHT (GM.)	REMARKS
	MILK	MEAT			
1916 Sept. 11 to 14	150	100	None	3,065	
Sept. 14 to 23	"	"	"	3,150	
Sept. 23 to Oct. 2	"	"	"	3,110	
Oct. 2 to 9	"	"	"	3,025	
Oct. 9 to 16	"	"	"	3,105	
Oct. 16 to 26	"	"	"	3,175	

PROTOCOL OF CAT 1 (BLACK AND BROWN)—CONT'D

DATE	DAILY FOOD WITH DRUG			WEIGHT	REMARKS
	MILK	MEAT	(GIVEN DAILY)	(GM.)	
Oct. 26 to Nov. 6	150	100	None	3,025	
Nov. 6 to 9	"	"	"	3,150	
Nov. 9 to 15	"	"	Silicotungstic acid, 2 ml. of 20% solution	3,100	
Nov. 15 to 22	"	"	"	3.165	
Nov. 22 to 30	"	"	"	3,143	Feces fluid for two days.
Nov. 30 to Dec. 4	"	"	"	3,145	
Dec. 4 to 11	"	"	"	3,141	Feces fluid for one day.
Dec. 11 to 18	"	"	"	3,114	Left about ½ food and drug on Dec. 9.
Dec. 18 to 26	"	"	"	3,165	Left about ½ on Dec. 12.
Dec. 26 to Jan. 4/17	"	"	"	2,883	Feces fluid for one day, left dose and milk on Dec. 28 and 29.
1917 Jan. 5 to 13	150	100	Potassium Silicotungstate, 4 ml. of 10% sol.	2.794	Left milk on Jan. 9 and 10.
Jan. 13 to 19	"	"	"	2,644	Left milk on Jan. 14.
Jan. 19 to 30	"	"	"	2,692	Left milk on Jan. 28.
Jan. 30 to Feb. 7	"	"	"	2,635	Left milk on Feb. 2.
Feb. 7 to 15	"	"	"	2,567	Left milk on Feb. 7.
Feb. 15 to Mar. 1	"	"	"	2,015	Left milk on Feb. 15, 17 and 23.
Mar. 1 to 9	"	"	"		Left some of food and drug daily.
Mar. 9 to 28	"	"	None	2.430	Left a little milk occasionally.
Mar. 28 to Apr. 8	"	"	"	3.470	
Apr. 8 to 14	"	"	"	3,357	

Cat No. 1 received 22.40 gm. of silicotungstic acid and 25.20 gm. of potassium silicotungstate, or a total of 47.60 gm. of silicotungstate in the course of 17 weeks. During this period the animal lost about one-third of its weight; but otherwise did not seem to be markedly affected. This loss of weight can hardly be attributed entirely to refusal of food, as this did not occur to a sufficient extent to explain it. Hence there probably occurred either a digestive or a metabolic disturbance, which became only marked during the second half of the feeding period; when even though potassium silicotungstate was given, no change in the downward tendency occurred. That the disturbance was not of a permanent nature, may be seen from the fact that when the drug administration was stopped, the cat rapidly regained and exceeded the weight it had prior to the drug feeding.

PROTOCOL OF CAT NO. 2 (GRAY STRIPED)

DATE	DAILY FOOD WITH DRUG			WEIGHT	REMARKS
	MILK	MEAT	(GIVEN DAILY)	(GM.)	
1916 Oct. 20 to 26	150	100	None	2,385	
Oct. 26 to Nov. 6	"	"	"	2,565	
Nov. 6 to 9	"	"	"	2,545	
Nov. 9 to 15	"	"	Silicotungstic acid 2 ml. of 20% sol.	2,620	
Nov. 15 to 20	"	"	"	2,623	Feces fluid for 2 days.
Nov. 20 to 29	"	"	"	2,640	
Nov. 29 to Dec. 4	"	"	"	2,675	
Dec. 4 to 11	"	"	"	2,711	
Dec. 11 to 18	"	"	"	2,931	
Dec. 18 to 26	"	"	"	2,900	
Dec. 26 to Jan. 6, 1917	"	"	"	2,673	Left about ½ of dose and food on Dec. 28 and 29.
Jan. 6 to 13	150	100	Silicotungstic acid, 2 ml. of 20% sol.	2,511	Left all of dose and milk on Jan. 4 and 5.
Jan. 13 to 19	"	"	"	2,289	Left dose and milk on Jan. 9 and 11.
Jan. 19 to 30	"	"	"	2,232	Left dose and milk on Jan. 20 and 28.

PROTOCOL OF CAT NO. 2 (GRAY STRIPED)—CONT'D

DATE	DAILY FOOD WITH DRUG			WEIGHT	REMARKS
	MILK	MEAT	(GIVEN DAILY)	(GM.)	
Jan. 30 to Feb. 7	150	100	Silicotungstic acid, 2 ml. of 20% sol.	2,260	Left dose and milk on Feb. 3 and 7.
Feb. 7 to 15	"	"	"	2,300	Left dose and milk on Feb. 9.
Feb. 15 to Mar. 1	"	"	"	2,520	Left food and drug daily. Animal seems depressed.
Mar. 1 to 9	"	"	"		Animal is sick.
Mar. 9 to 28	"	"	None	2,430	Left a little milk occasionally.
Mar. 28 to Apr. 8	"	"	"	2,712	
Apr. 8 to 14	"	"	"	2,916	

PROTOCOL OF CAT NO. 3 (GRAY STRIPED—MALE)

DATE	DAILY FOOD WITH DRUG			WEIGHT	REMARKS
	MILK	MEAT	(GIVEN DAILY)	(GM.)	
1916 Oct. 16 to 26	150	100	None	2,265	
Oct. 26 to Nov. 6	"	"	"	2,260	
Nov. 6 to 9	"	"	"	2,400	
Nov. 9 to 15	"	"	Silicotungstic acid 5 ml. of 20% sol.	2,510	
Nov. 15 to 22	"	"	"	2,416	Liquid bowel movements Nov. 18 to 22.
Nov. 22 to 29	"	"	"	2,398	
Nov. 29 to Dec. 4	"	"	"	2,345	Liquid bowel movements Dec. 2 to 4.
Dec. 4 to 12	"	"	"	2,300	Ate about half Dec. 4 and 12.
Dec. 12 to 18	"	"	"	2,280	Liquid bowel movements, Dec. 14 and 15.
Dec. 18 to 26	"	"	"	2,339	Liquid bowel movements, occasionally.
Dec. 26 to Jan. 3, 1917	"	"	"	2,310	Left most of drug and food after Dec. 29
Jan. 3, 1917	"	"	"		Animal weak and sick. Died 3 P.M.

Cat No. 2 received 47.60 gm. of silicotungstic acid, mixed with its milk, in the course of 17 weeks. During the first seven weeks, the animal gained in weight. During the remainder of the period, the animal left more and more of its food as the time went on, until during the last week it ate very little. At this time the animal seemed quite sick; and it probably would have died if the drug feeding had been continued. To see whether permanent damage was done, the drug feeding was stopped; and complete recovery occurred.

Necropsy Report by Dr. D. J. Davis (Univ. of Ill.).—Extensive hemorrhage in anterior mediastinum and in subpericardial tissue, extending toward visceral pericardium.

Hemorrhage, 2 cm. in diameter, in middle lobe of right lung, involves entire thickness of lung and extends to hilus of the three lobes. Microscopic examination shows the alveoli uniformly filled with blood. The walls are usually distinct, but here and there are broken through; occasionally large regions of hemorrhage appear, containing quite fresh blood. Bronchioles also contain blood. The pleura is normal.

Heart muscle flabby and grayish, small hemorrhage in wall of right auricle, and small subpericardial hemorrhage on left ventricle. Valves, cordæ tendineæ and intima of large vessels, bile stained. Myocardium shows no alterations on microscopic examination.

Liver enlarged, flabby, soft, grayish yellow, greasy on section. Gall bladder moderately distended with yellowish green bile. Microscopically the liver lobules are found to contain large amounts of fat, evenly distributed. At the periphery are deposits of granular biliary pigment. The central veins are normal in appearance. Kupffer's cells contain large quantities of yellow pigment granules.

Spleen slightly pale in color and mottled in places. Pancreas grayish white and soft. Neither spleen nor pancreas reveals microscopic changes worthy of note.

Stomach, negative on gross examination. Microscopically there are found in the mucosa, here and there, small hemorrhages; and, on the surface, rather extensive regions of necrosis in which numerous blood cells appear.

Small intestine, 10 inches above ileo-cecal valve shows submucosal hemorrhages, becoming more frequent and extensive higher up to 10 inches below the stomach. Colon negative.

Kidneys.—Capsule strips readily, leaving a smooth surface. Cortex yellowish gray. Medulla quite red. Markings very distinct. Microscopic examination: The cells of the convoluted tubules appear somewhat swollen and the lumen of the tube is frequently filled with granular detritus. Deposits of rather fine yellow granules appear, especially in the cells of the collecting tubules; also here and there throughout the cortex. The glomeruli are normal in appearance, as is also the capsule.

Urine from bladder contains bile, albumin, no sugar, a few casts, and bile-stained leucocytes and epithelial cells. Some spermatozoa.

Summary.—Cat No. 3 consumed at least 50 gm. of silicotungstic acid, mixed with milk, in the course of about two months, before death ensued from fatty degeneration of the liver with multiple hemorrhages in various organs and a

moderate degree of nephritis. The necropsy findings remind one somewhat of those of phosphorus poisoning.

Summary of the Feeding Experiments

From the above detailed experiments, it seems evident that silicotungstic acid or potassium silicotungstate may be fed to animals for considerable periods and in fairly large quantities. A dose of 50 gm. of silicotungstic acid, consumed in about 8 weeks, killed cat No. 3, while somewhat less than this quantity (47.60 gm.), given in 17 weeks, was survived by cats Nos. 1 and 2. Hence silicotungstic acid, given by mouth, is not very toxic to cats.

THE ACTIVITY OF DOUBLE TUNGSTATES OF ALKALOIDS

Strychnine Phosphotungstate.—This compound, kindly furnished me by the Abbott Laboratories of Chicago, was administered to two dogs with results shown by the following protocols of the experiments:

May 29, 1916. Dog 1.—Weight 4800 gm. Not fed for 24 hours.
8:35 A.M. Strychnine Phosphotungstate 0.038 gm. (= 8 mg. per kg.). Administered per stomach tube.
8:52 " Violent convulsion.
9:12 " Convulsive starts.
10:30 " Convulsion.
11:30 " Convulsion.
1:15 P.M. Dead.
June 1, 1916. Dog 2.—Weight 4500 gm. Just fed.
9:15 A.M. Strychnine phosphotungstate 0.036 gm. (=8 mg. per kg.). Administered by stomach tube.
9:45 " Convulsion.
10:15 " Convulsion.
11:40 " Dog standing up. Seems better.
1:30 P.M. Dead.

From these experiments, it will be seen that 8 mg. of strychnine phosphotungstate is fatal to dogs in about 4 hours, convulsions appearing in 16 minutes when the stomach was empty and in 20 minutes when the stomach was full of food. The difference in the condition of the stomach in these two cases evidently made but little difference in the results. Inasmuch as strychnine phosphotungstate represents about 25 per cent of the alkaloid, the dose used corresponds to 2 mg. of strychnine per kg., which is a fatal dose for the dog as will be seen from the following experimental protocols:

Dog A 1.—Weight 8500 gm. was given 0.017 gm. strychnine sulphate (= 2 mg. per kg.). Convulsions in 12 minutes, death in 26 minutes.

Dog A 2.—Weight 9500 gm. was given 0.019 gm. strychnine sulphate (= 2 mg. per kg.). Convulsion in 10 minutes, death in 11 minutes.

The chief difference, it will be noted, is in the time of the occurrence of death, which was an average of about 18 minutes with strychnine sulphate, and 4 hours with strychnine phosphotungstate. Our results with this compound agree with those of Nielsen.[1]

Strychnine Silicotungstate [SiO_2, $12WO_3$, $2H_2O$. $4C_{21}$ H_{22} N_2 O_2 + 8 H_2O *(Bertrand)*]

Inasmuch as Strychnine Silicotungstate is less bitter than the phosphotungstate, it was subjected to tests. It was prepared by mixing boiling solutions of 2 moles of strychnine sulphate $(C_{21}\ H_{22}\ N_2O_2)_2\ H_2SO_4$ + 5 H_2O (by weight one part) with 1 mole of potassium silicotungstate—2 K_2O, SiO_2, 12 WO_3 18 H_2O (by weight two parts). The compound prepared by mixing cold solutions was slightly more bitter than that prepared by the hot process.

That strychnine silicotungstate is highly active, is shown by the following protocols of experiments.

Pup 1, weight 2190 gm., was given 17.5 mg. of strychnine silicotungstate (= 2 mg. of strychnine per kg.) by means of the stomach tube. Convulsions in thirty minutes, death in forty minutes.

Pup 3, weight 1900 gm., was given 15.2 mg. of strychnine silicotungstate (= 2 mg. of strychnine per kg.) by stomach tube. Convulsions in thirty minutes, death in forty minutes.

Cat 1, weight 2670 gm., was given 21.4 mg. strychnine silicotungstate (= 2 mg. of strychnine per kg.) by stomach tube. Convulsions in thirty-five minutes, death within two hours.

Cat 2, weight 800 gm., was given 6 mg. strychnine silicotungstate (=2 mg. per kg. of strychnine) by stomach tube. Convulsion in twelve minutes, death in twenty minutes.

Cat 3, weight 3850 gm., was given 30.8 mg. strychnine silicotungstate (= 2 mg. per kg. of strychnine) by stomach tube. Convulsions in fifteen minutes, death in twenty-five minutes.

To compare the activity of strychnine silicotungstate with that of strychnine sulphate, the minimal lethal dose of each was determined by experiments upon rabbits, with the result shown in Table III.

TABLE III

COMPARATIVE TOXICITY OF EQUIVALENT DOSES OF STRYCHNINE SULPHATE AND OF STRYCHNINE SILICOTUNGSTATE TO RABBITS

STRYCHNINE SULPHATE				STRYCHNINE SILICOTUNGSTATE			
Dose mg. per kg.	No. of Animals	Results Conv's	Final	Dose mg. per kg.	No. of Animals	Results Conv's	Final
				10	1	In 5 hours	Recov'd
5	6	In 35 min.	3 died	15	3	In 4½ hours Av.	All Recov'd
				20	8	5½ hours Av.	5 died

It will be seen that the lethal dose of 5 mg. of strychnine sulphate equaled in its effects the dose of 20 mg. of strychnine silicotungstate. Inasmuch as 3 mg. of strychnine silicotungstate represents the same amount of strychnine that is contained in 1 mg. of strychnine sulphate, the relation of toxicity is as 5

to 6.6, a difference that is certainly not great. It is of interest to note the long time that elapsed in the strychnine silicotungstate experiments in the rabbit before convulsions appeared. This is in marked contrast to the comparatively prompt appearance of convulsions in cats and in dogs, in which animals convulsions occur so soon after administration of the dose that it is hard to believe that absorption did not occur from the stomach.

Double Tungstates of Emetine

I prepared emetine silicotungstate, a salmon-colored tasteless powder, by mixing 1 part of emetine hydrochloride with 2.7 parts of potassium silicotungstate. This was compared with emetine phosphotungstate (furnished by the Abbott Laboratories), 3.7 parts of which equal 1 part of emetine hydrochloride. It will be seen that these two preparations are of the same strength.

Experiments upon Mice.—White mice were fed with cakes made of cracker, sugar and water to which a definite amount of the agent to be tested was added. The results are shown in the following table. (Table IV.) The animals that died succumbed within 24 to 48 hours.

TABLE IV

COMPARATIVE TOXICITY OF EQUIVALENT DOSES OF EMETINE PHOSPHOTUNGSTATE AND OF EMETINE SILICOTUNGSTATE FOR WHITE MICE

DOSE PER 20 GM. MOUSE	EMETINE PHOSPHOTUNGSTATE		EMETINE SILICOTUNGSTATE	
	No. of Animals	Results	No. of Animals	Results
2.5 mg.	2	Recovered	3	Recovered
5 mg.	3	"	2	1 died
10 mg.	3	All died	1	died

It will be seen that the emetine double tungstates are both highly and probably approximately equally toxic. Owing to the comparative innocuousness of the tungstates, as shown by our feeding experiments, the toxicity must be ascribed to the emetine.

A few experiments were performed upon cats with the following results:

Cat. 1.—Weight 2700—Aug. 3/16.

10:00 A.M. 1 gm. Emetine Phosphotungstate by stomach tube.
11:45 " Emesis.
P.M. Slight emesis.
Defecation.
Recovered.

Cat 2.—Weight 3700—Aug. 5/16.

10:45 A.M. 0.5 gm. Emetine Phosphotungstate by stomach tube.
Did not vomit.
No other symptoms.

Cat. 2.—Weight 3700—Aug. 14/16.

10:30 A.M. 1 gm. Emetine Silicotungstate by stomach tube.
1:30 P.M. Large bowel movements. No emesis.
No other symptoms.

These results agree, on the whole, with those of Nielsen who showed that,

while small doses of emetine phosphotungstate did not produce emesis, large doses did; though not nearly to the same extent to which the same dose of emetine hydrochloride would have acted.

Quinine Silicotungstate

That this combination, which may be prepared by precipitating a solution of 1 part of quinine hydrochloride with 2.26 parts of potassium silicotungstate, is not very toxic to a small dog, may be seen from the following experimental protocol:

PROTOCOL OF PUP 3.—FEMALE—BLACK AND WHITE

DATE				WEIGHT
Aug. 3				1550
" 7	Quinine Silicotungstate	0.5 gm. by	Stomach Tube	1710
" 8	"	"	"	
" 9	"	"	"	1590
" 10	"	"	"	1550
" 11	"	"	"	1535
" 12	No dose			
" 13	"			
" 14	Quinine Silicotungstate	"	"	1700
" 15	"	"	"	1780
" 16	"	"	"	1780
" 17	"	"	"	1730
" 18	"	"	"	1830
" 19	"	"	"	1920

This animal received 5.5 gm. of quinine silicotungstate in the course of 11 days without the production of unfavorable results, excepting an initial loss of weight, which was rapidly recovered from in spite of continuance of the administration.

That quinine silicotungstate is capable of producing effects, may be seen from the following typical protocol of a mouse experiment:

PROTOCOL OF MOUSE 2

DATE	FED CAKE COMPOSED OF		WEIGHT	REMARKS
		gm.		
Aug. 2	Quinine Silicotungstate	0.10	15 gm.	
	Cracker	2.00		
	Sugar	0.50		
	Water	1.00		
" 3	Same dose		16 "	
" 4	" "		16 "	
" 5	" "		16 "	Feces greenish gray.
" 6	" "			Left some.
" 7	" "		14½ "	
" 8	" "		15 "	Ate little.
" 9	Dead			

This animal consumed, at least, 0.50 gm. of quinine silicotungstate in the course of 7 days before death occurred. That the result was due to the drug is rendered evident by the fact that control animals did well on the food without

the drug and that similar results were obtained with three other mice, whose protocols might be summarized as follows:

Mouse 1.—Consumed 0.5 gm. quinine silicotungstate in daily amounts of 0.05 gm. in the course of 12 days, before death occurred.

Mouse 3.—Consumed 0.3 gm. quinine silicotungstate in daily amounts of 0.10 gm. in the course of 3 days, before death occurred.

Mouse 4.—Consumed 0.6 gm. quinine silicotungstate in daily amounts of 0.10 gm. in the course of 6 days, before death occurred.

It may be of interest here to note that, while mice refused to eat cakes made with quinine sulphate or with potassium silicotungstate, they readily ate cakes made with quinine silicotungstate, evidently because of its comparatively slight bitterness.

CONCLUSIONS

1. The comparative tastelessness and slight local effects of phosphotungstates and silicotungstates of alkaloids might make these combinations therapeutically useful.

2. The phosphotungstate and silicotungstate radicals are sufficiently nontoxic to be used for the administration of alkaloids with small dosage, such as strychnine or emetine.

3. Inasmuch as the tungstates, when taken in liberal amounts for a long time, do have a toxic action, it is probably not advisable to use these combinations for the administration of quinine or other alkaloids that would be given in large doses, until further studies of the toxicity of these have been made.

BIBLIOGRAPHY

[1]Nielsen, Carl: The Alkaloidal Phosphotungstates, Am. Jour. Clin. Med., Nov., 1916, 903.
[2]Krüger, Richard: Kolloidales Wolfram als Ersatz für Wismut bei Roentgenaufnahmen des Magen—und Darmkanals, München med. Wchnschr., 1912, lix, 1910.
[3]Bernstein-Kohan, Jacob: Über die Wirkungen des Wolfram's, Arb. d. Pharmk. Inst. zu Dorpat., Stuttgart. 1890, v, 42-126.
[4]Kobert, R.: Lehrbuch der Intoxicationen, Stuttgart, 1906, 323.
[5]Kunkel, A. J.: Handbuch der Toxicologie, Gustav Fischer, Jena, 1899, 221.
[6]Gmelin-Kraut, iii, 1, 856.

THREE CASES WHICH ILLUSTRATE THE CONSEQUENCES OF CORONARY LESIONS*

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CASE I.

J. B., Hospital No. 1905, a colored man 39 years old, was admitted to the Cincinnati General Hospital on May 8, 1915, complaining of a sharp pain under the sternum.

According to the Clinical Notes, the *family history* was irrelevant.

Past History.—The patient had measles and mumps in infancy. Ten or fifteen years ago he had smallpox; and, twelve years ago, rheumatism. About fifteen years ago he had pneumonia. Seven years before admission he had gonorrhea, and three years before that he had a bubo. He does not smoke or chew, and has used no alcohol since January 1, 1915.

Present Illness.—This commenced in July, 1914, when he began to experience dyspnea when working hard or climbing a hill. He had a feeling of fullness in the chest. In February, 1915, he began to have pain in the sternal region. On admission, this pain was described as being of a dull aching sort which, at times, becomes quite sharp. It has forced him to stop work and rest for 10 or 15 minutes, after which he could return to work. For several days the patient has had a cough.

Present State.—The patient is a well-developed, and well-nourished colored man. Temperature 100°; pulse 128; respirations 40. General condition fair. The pupils are equal and react to light. The conjunctivæ are muddy, the tongue clean, the teeth and gums "poor." The epitrochlear glands are palpable. The chest is symmetrical. The right lung is resonant anteriorly, but shows a diminished posterior resonance on percussion. On auscultation there are crepitant rales at the left base posteriorly; the breath sounds are bronchial in character. The left border of the heart is normal. The apex is in the nipple line. The rhythm is regular. There is a diastolic murmur at the apex and a systolic in the aortic area. The pulses are small. The liver is enlarged.

May 9, 1915.—In the evening the patient fell out of bed and was found sweating profusely, with a complete left sided hemiplegia. The tongue deviated to the left, the left side of the face was smooth and the patient was unable to move the left side.

May 10, 1915.—At 9:30 A.M., all evidence of the previous evening's attack had disappeared.

May 20, 1915.—The patient complained of pain in the left chest on deep inspiration. There was roughened breathing over the left lower lobe and, on the right, between the scapula and the spinal column. The heart began to show some irregularity.

May 22, 1915.—The patient said the pain had shifted to the right lower chest and right hypochondrium. There was slightly prolonged expiration and rough breath sounds over the left base, posteriorly. Over the right lower posteriorly crepitant rales were heard. The patient is spitting flecks of blood, bright red in color.

June 2, 1915.—Wassermann reaction strongly positive.

June 20, 1915.—At 5:25 A.M., the patient got up, dressed, and went to the sun room. He complained of pain in the cardiac region, became dyspneic and died suddenly before the doctor could be reached.

Clinical Diagnosis.—Mitral regurgitation; mitral stenosis; pericarditis; passive congestion of the lungs and liver; syphilitic aortitis; chronic nephritis; coronary sclerosis(?) with thrombosis or embolism (?).

AUTOPSY PROTOCOL

The body of an extremely well-built, and well-nourished colored adult male, 6 feet long. There was nothing remarkable about the head except that the bridge of the nose was thick and flattened and somewhat larger to the right than to the left of the median line. Across the chest were three hypertrophic linear scars, one opposite the second inter-

*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

space, another, opposite the third, and the last, opposite the fourth interspace. These were all somewhat longer than the width of the sternum. The lower molars were absent on both sides. The third upper molar was loose in the gum; the second upper molar was carious; the three upper left molars were dead. There was a general pyorrhea alveolaris. There was a perforation of the nasal septum. The peripheral lymph glands were not demonstrably enlarged. The testicles were apparently normal. There was no definite preputial scar. The tibial margins were smooth. There was no postmortem rigidity except in the muscles of the jaw. There was no lividity. The body was still warm.

The left pleural cavity was completely obliterated. The right was completely obliterated antero-laterally and apically. In the upper portion was a collection of a straw-colored serous fluid (500 c.c.). There was a slight increase of pericardial fluid, and over the left ventricle anteriorly, posteriorly, and apically were recent fibrinous adhesions.

There was nothing abnormal about the position or appearances of the intestines. The tongue and posterior pharynx, larynx, esophagus and trachea, were apparently healthy. The tonsils were fibroid and atrophic. The lungs were markedly edematous and somewhat salmon-colored, and showed a mild grade, at least, of passive congestion. Edema was more evident in the left lung. There were two very small subpleural areas of consolidation in the right lung which resembled in form and color, incomplete infarcts. The pleura over them was roughened.

The heart was large and flabby. When it was opened, it appeared that it was markedly dilated, and only moderately hypertrophic. The walls were generally thickened, and not contracted apparently, and were gray and fatty. The valves, with the exception of the aortic, were not deformed, and the aortic only to a slight extent. The right and posterior cusps at their common attachment were adherent and slightly contracted. The papillary muscles were fibroid. The aorta was the seat of a diffuse luetic aortitis with evidence of all stages of the process from fatty degeneration, and patchy opalescent succulent fibrosis, to atheroma and calcification. It was very obviously dilated to perhaps one-half more than its normal size. The mouths of the coronaries were remarkably constricted so that the finest probe could be passed with difficulty into the left and could just be passed easily into the right. The coronaries themselves were not sclerotic. The pericardium, over the left ventricle, was congested, roughened, and covered with a fibrinous exudate, beneath which were numerous small hemorrhages.

The spleen was of moderate size, congested, and somewhat fibrotic. The kidneys were enormously congested, and almost purple in color. The capsules removed with ease, tearing in but a few places. There were a few small retention cysts beneath the capsules and the stellate veins were congested. The cortex was of about normal thickness in the left, while in the right it was increased. The surfaces of both organs were somewhat finely granular. The liver was not especially abnormal. It was congested and apparently fatty. The surface was smooth. The cut surface was distinctly fatty and had an indistinct nutmeg appearance. The gall bladder held a fair quantity of yellowish-green mucoid bile. The ducts were patent. The pancreas and adrenals were not remarkable. The stomach and intestines were congested, and in the ileum the solitary follicles were enlarged, especially near the cecum where they were congested and quite raised above the surface. There were patches of congestion in the large intestine. The appendix was normal.

The prostate and bladder were not abnormal.

Anatomic Diagnosis.—Syphilitic aortitis involving the mouths of the coronaries, especially the left; cardiac dilatation; passive congestion and edema of the lungs, and abdominal organs; chronic diffuse nephritis; acute follicular enteritis; acute fibrinous pericarditis; pleural effusions; luetic perforation of the nasal septum; pyorrhea alveolaris.

REMARKS

In this case, a luetic process in the aorta affected the tisues just about the coronary openings and gradually led to stenosis of these vessels. This process went on for several months during which time the heart gradually lost its reserve, so that exercise caused myocardial insufficiency, and later dilated so that the valvular orifices dilated and at them murmurs appeared. Finally it happened that while the narrowed coronaries permitted just enough blood to pass to nourish the myocardium while the patient was quiet, not enough was able to pass to nourish

the muscle during even slight exertion; and cardiac failure and death appeared.

In this case there was a general cardiac dilatation which means, of course, a general lack of blood to the myocardium.

CASE II.

E. G., Hospital No. B-818, a white man, 77 years old, was admitted to the Cincinnati General Hospital on Feb. 3, 1917, complaining of a "sore leg."

The family history was negative.

He denied venereal disease. He has been married 30 years, and has had 3 children. His wife had no miscarriages.

The present condition dates from the first of the year when both lower legs became, gradually, swollen and red, and walking became difficult and painful.

On admission the patient appeared as an old well-developed and well-nourished man with a temperature of 97, pulse 68, and respirations 24. The tongue was foul and covered with a mucopurulent material. The gums were "poor." The lungs appeared normal. The cardiac rhythm was regular, and the sounds were faint and weak. The abdomen was flat. Upon both lower legs there were large infected ulcerations which were somewhat elevated and very hyperemic. The urine was negative.

The next note made two weeks later says that the condition was improved. A week later the patient was stuporous. There was no further note on the case.

Death occurred on March 14, 1917.

Clinical Diagnosis.—Dermatitis.

AUTOPSY PROTOCOL (H).

The body was that of a fairly well-nourished, well-built, white man of apparently 80 years. The pupils were equal. Many of the teeth were missing and some of those which remained were decayed. The superficial lymph glands were not appreciably enlarged. The chest was deep and barrel-shaped. Upon the lower two-thirds of the right leg was an area over which the skin was sloughed and upon which there were numerous small ulcers. In a corresponding situation on the left leg was a similarly ulcerated but smaller area of dermatitis.

The body cavities contained no fluid. The mesentery and omentum were well supplied with fat. The appendix was *in situ* and evidently healthy. When the sternum was removed the lungs did not collapse chiefly because of emphysema, for in all other respects these organs were normal, except that in the upper portion of the left lower lobe there was a small infarct, the base of which measured 2 cm. in diameter.

The heart was enlarged to fully twice its normal size. Fully three-fourths of the enlarged organ was occupied by the left ventricle. Nevertheless, all the orifices were dilated. There were no essential valvular changes. The left ventricle was filled in its apical half by a large, partially firm mass of adherent red clot which was attached most firmly to the anterior ventricular wall which was about one-third the normal thickness. Also, externally, the anterior left ventricular surface presented an irregularly shaped softened congested area with rather sharp outlines which represented a myocardial area supplied by the descending branch of the left coronary. Dissection of the coronaries showed that they were generally sclerotic. The left coronary was somewhat narrowed but not exceedingly so, while the descending branch was so sclerotic and so nearly occluded that the finest probe could not be made to pass. The myocardium was generally pale and showed scattered areas of fatty degeneration. The papillary muscles were exceedingly fibrotic. The aorta showed generally distributed fatty patches and white, glistening, firm, fibrotic areas, associated with linear intimal wrinkling. There were also numerous calcified plaques, especially in the abdominal segment.

The liver was small and congested. Section showed the nutmeg appearance of a passive congestion associated with an evident diffuse fibrosis, and some fatty changes. The spleen was not unusual though it was congested. It was of the normal adult size and therefore enlarged.

The kidneys were embedded in large amounts of perirenal fat. The capsules stripped with fair ease leaving somewhat congested, slightly granular, sclerotic surfaces. The venules were prominent. Upon section the parenchyma was decidedly congested. The cortices and medullæ were faint. There was evidently a patchy fatty degeneration of the cortical parenchyma.

The bladder contained a small amount of turbid urine. The prostate was somewhat enlarged. There was nothing remarkable in the gastrointestinal tract or pancreas.

Anatomic Diagnosis.—Coronary arteriosclerosis and thrombosis; myocardial infarction; cardiac dilatation; myocardial fibrosis and fatty degeneration; aortic atherosclerosis (luetic?); passive congestion of the abdominal viscera; chronic diffuse nephritis; pulmonary emphysema and infarction; ulcerative dermatitis.

Fig. 1.—Case B. 818. Infarction of the papillary muscle. Myocardial fatty degeneration.

Fig. 2.—Case B. 818. Cardiac hypertrophy and dilatation; ventricular aneurysm (partial); ventricular thrombosis; myocardial fatty degeneration.

REMARKS

It seems a pity that the clinical notes in this case are not of some value. Nevertheless the heart illustrates the effect of a localized lack of blood in the myocardium, in contrast with the effects exhibited in Case I.

Here, although there was some general coronary sclerosis, there was little general myocardial disturbance, but in the region supplied by the descending

branch of this vessel, the muscle was badly damaged. The appearances suggest that the sclerotic process had been progressing for some considerable period of time during which the vessel was constricted and also distorted by atheroma. They also suggest that, terminally, a thrombus was formed in the atheromatous portion and that this quite suddenly blocked the vessel and so led to the appearance of the infarct. That there was some time elapsing between the infarction and the older process is suggested by the dilatation of the area of myocardium in which the infarct appeared and by the age of the endocardial portions of the interventricular clot. The lack of blood supply was tending to the production of a cardiac aneurism, a process which was terminated by myocardial death and complete cardiac failure.

CASE III.

E. W., Hospital No. 6774, a colored woman, 37 years of age, was admitted to the Cincinnati General Hospital on Dec. 21, 1915, complaining of "pain across the chest."

Family History.—Negative.

Past History.—The patient had been a healthy child. She had the usual diseases of childhood and has had many attacks of sore throat, accompanied by rheumatic pains; but she gave no history of inflammatory rheumatism. Menstruation began at 16 and since then has been accompanied by marked dysmenorrhea. At the age of 32, the menstrual flow became prolonged and excessive, and at 33, a panhysterectomy was done. The patient says a tumor was removed. Since the operation she has been well, though each day she has had to take a cathartic. She has had no children and no miscarriages.

Present Illness.—Three weeks before admission, the patient had a gastric upset the cause of which she does not know. This was characterized by vomiting, pain in the chest, headache, and general weakness. It was because of these symptoms that the patient came to the hospital.

Present State.—The patient lies quietly in bed and has rapid, shallow respirations, and no movement of the alæ nasi. The pupils are equal and react to light and during accommodation. The gums are slightly pyorrheic. The throat and tonsils are reddened, but upon them there is no exudate. The anterior cervical glands are palpable. The chest is symmetrical, though expansion is slightly greater on the right than on the left. The vocal fremitus is more marked on the right than on the left. On percussion the note over the left apex and infraclavicular space and downward is higher pitched than on the right. Auscultation is entirely negative. The apex beat is neither seen nor felt. The relative cardiac dullness reaches 10.5 cm. to the left in the fifth interspace, and 2.5 cm. to the right in the fourth. There is no abnormal retrosternal dullness. In the apex region a soft, blowing, systolic murmur is heard, well transmitted to the axilla. The second aortic is louder than the second pulmonic. The spleen and liver are not felt. Over the lower right quadrant there is an increased resistance and the patient complains of some tenderness in this region.

December 22.—The patient says there is no pain in the chest, though there is marked epigastric tenderness. There is muscle spasm in the upper half of each rectus. The respiratory movements are free. Auscultation over the heart shows a leathery scratchy friction rub heard between the first and second sounds. This is loudest in the fifth interspace between the midsternal and parasternal lines, and is heard as high as the third rib. The second sounds at the base are both accentuated and of about equal intensity. Following the second sound there is a soft systolic murmur heard best in the second and third interspaces on the left. The friction rub is not palpable. The abdominal pain is probably referred diaphragmatic. The apex of the heart is palpable beneath the fifth rib. The relative cardiac dullness is the same as on admission. The cardiohepatic angle is 90°. The pulse is regular, of moderate size, and rather quick. No capillary pulse is seen. The friction rub does not disappear with deep breathing, and is present when the patient holds her breath. (At 5 P.M., the friction rub *had* disappeared and was never heard again.)

December 23.—Systolic and diastolic cardiac murmurs are heard. The patient has been nauseated and has vomited frequently.

December 27.—Vomiting has disappeared for several days. The pulse rate has slowed.

The size of the heart has remained unchanged and the other phenomena are still present. Blood pressure, systolic 140, diastolic 92.

On admission, the urine was negative. Later it showed finely granular casts. A blood culture was negative on Dec. 31. On admission, the leucocyte count gave 14,200, of which 85 per cent were neutrophils. Hemoglobin, 85 per cent. Later the leucocyte count became normal and remained so. A Wassermann test was positive.

During the hospital period, the patient had several attacks of tachycardia.

The first two days in the hospital, the pulse ran between 120 and 150. The temperature was never above 100.5°. The temperature and pulse became normal and remained so until Jan. 12, when the pulse gradually began to rise to 110. On Jan. 18, the pulse rose to 160 and the temperature to 100.5°. The pulse then gradually returned to normal.

Clinical Diagnosis.—Coronary thrombosis (?); syphilis; syphilitic aortitis; aortic insufficiency (relative); healed fibrinous pericarditis.

AUTOPSY PROTOCOL

The body was that of a well-nourished, well-built colored woman of about 30 or 35 years of age and 5 feet, 3½ inches tall. There was a very slight edema of the ankles. The teeth were in excellent condition for the most part. The pupils were equal; rigor mortis was absent; postmortem lividity was practically absent. Upon the abdomen, running from the umbilicus to the pubes, was a healed linear scar, 15 centimeters long. Over the chest, extending from nipple to nipple and measuring 9 cm. in width, was an area of pigmentation similar to that produced by the application of a mustard plaster. The finger nails were pale. There were no external signs of traumata. The subcutaneous fat was well developed. In the abdomen was a small amount, a few hundred centimeters, of a rather clear serous fluid. There were numerous old veil-like adhesions between the anterior surface of the liver over both lobes and the parietal peritoneum. One loop of ileum and the omentum were adherent to the anterior abdominal wall just below the umbilicus in the region of an old surgical incision. The liver was enlarged and reached to a point 14 centimeters below the ensiform. The peritoneum covering the intestines, and the parietal peritoneum were smooth and shiny, and showed no evidence of inflammatory changes. The appendix was present, 9 cm. long, and, from its beginning, ran directly upward along the posterior surface of the cecum. The stomach was moderately dilated and had an hour-glass shape. When the sternum was removed, there was a gush of clear serous fluid from each pleural cavity, and the lungs did not collapse. There were a few old adhesions in the upper part (posterior) of the left pleural cavity, none in the right. When the bronchi were cut, it was found that there was a large amount of fluid in them. The mediastinal tissues, especially at the hilum of the lungs, were edematous. There was very little pigmentation, anthracosis, and no congestion. There was crepitation throughout. There was a small amount of clear straw-colored pericardial fluid. The blood was fluid. The omentum was adherent to the posterior part of the wall of the pelvis over the rectum and the sigmoid formed a very sharp long loop just before it entered the pelvis. Behind this loop of sigmoid, the small intestine ran and was adherent to the sigmoid low in the pelvis. The mesenteric lymph glands were moderately hyperplastic. There were old adhesions between the cardiac portion of the stomach and the left lobe of the liver. The uterus, both tubes, and ovaries were missing. The bladder was small and contracted. The stomach was filled with partially digested food. The mucous membrane of the stomach was covered with a thick layer of rather tenacious mucus. There was no evidence of ulceration or scar on the internal surface of the stomach. The duodenum showed no obvious abnormality. The pancreas was not abnormal.

The heart was enlarged, the hypertrophy affecting particularly the left ventricle. The tricuspid orifice admitted the passage of three and a half fingers. The pulmonary orifice was apparently normal. The mitral admitted two fingers; the aortic admitted one and a half fingers. The aortic leaflets were only very slightly thickened at the edges, but the whole aortic region immediately above the valves was tremendously thickened, contracted, sclerotic, and just above the junction of the right and left leaflets, there was an area which resembled true bone, within the aortic wall. This area measured 9 mm. in thickness and there was some evidence, to the eye, of marrow formation in this. The mouth of the right coronary was very much contracted so that only the tip of a fine probe was admitted. The mouth of the other coronary also was contracted, though not to the same degree. The whole arch of the aorta was the seat of a typical syphilitic mesaortitis, with very little calcification but a fair amount of fatty degeneration. The coronaries themselves were not sclerotic. There was no macroscopic evidence of thrombosis or embolism. The walls of

the left ventricle were thickened, firm and contracted. The myocardium showed evidence of fibrosis and also of parenchymatous changes which were particularly well seen in the papillary muscles in which there was evidence of a considerable amount of fatty change. The left papillary muscle appeared to be the seat of an almost complete myomalacia superimposed upon a fibrosis. The other valves of the heart were apparently normal.

The liver, 1280 grams, was flabby, congested and gray. These changes, together with the peripheral adhesions, were the only abnormalities in this organ. The gall bladder was filled with a thin brownish mucoid bile. The ducts were patent. There were no calculi. The spleen was of about normal size. The capsule was slightly and irregularly thickened, and running along the anterior edge, 1 cm. from the edge, was a broad linear scar that had caused a puckering in the organ. On section, the pulp was moist and of good color. The malpighian bodies were not visible. The left kidney was slightly smaller than normal, the capsule was somewhat adherent, though it removed with fair ease and tore the cortex in but a few places. The surface was paler than normal. The cortex was somewhat thinned, as was also the medulla. The line of demarcation between the cortex and medulla was not brilliant. There was moderate congestion of both cortex and medulla with evidence in linear striæ of fibrosis of the pyramids. The right kidney was a little larger, 175 grams, than the left, but the general appearances were the same. The lungs were voluminous, pale, crepitated throughout, and were soggy. The pleural surfaces were smooth except that of the apex of the lower left lobe where there was a mass of tags of old adhesions. There were no adhesions between the lobes. The apices were not scarred. On section, the tissue seemed to be saturated with fluid which dripped from the cut surface. Edema was very well marked. The adrenals showed no obvious changes.

Anatomic Diagnosis.—Syphilitic mesaortitis; contraction of the coronary orifices; myocardial fibrosis and degeneration; cardiac hypertrophy and dilatation; slight chronic nephritis; catarrhal gastritis; hystero-oophorectomy (old).

REMARKS

The progress of events in this case may have been somewhat as follows:

As the result of the development of a specific infection which affected, as it usually does, both myocardium and aorta, both became fibrotic and less elastic. As a result of this, particularly of the lack of elasticity, the myocardium underwent hypertrophy. Later, the aortic sclerosis developing about the mouths of the coronaries began to narrow them to such an extent that gradually the supply of blood to the myocardium was reduced to a point when, during exertion, was not sufficient for the needs. Transient myocardial failure resulted, but this passed away with rest. Finally, the coronaries became so diminished in size that even in the absence of exertion not enough blood reached the myocardium and it failed permanently.

SUMMARY*

These cases, taken together, illustrate the effects of gradual cutting down of the blood supply to the myocardium. In two cases the process was diffuse, slow and gradual, and led to general ventricular dilatation. In the other, the process was limited to one coronary and, in particular, to one branch. During a certain period the result was gradual though localized, dilatation tending to the production of a cardiac aneurysm. In the final period the process was abrupt and produced an infarct.

*Other coronary disease sequels have been commented upon in a former paper on Cardiac Aneurysms, in this journal, 1917, ii, 221.

LABORATORY METHODS

THE GERMICIDAL VALUE OF THE COMMON GYNECOLOGIC DOUCHING AGENTS*

By J. R. Stark, M.D., Cincinnati, Ohio

THE prevailing skepticism displayed as to the intrinsic germicidal value of the common gynecologic douching agents has prompted this series of experiments, wherein the solutions were used under conditions approaching as nearly as possible those of the human body.

So far as could be ascertained, the method employed in these investigations is original. The attempt was made to avoid complicated methods, to omit such phases as the Rideal-Walker phenol coefficient, etc. The douches employed at the Cincinnati General Hospital and commonly in use in private practice were used in this instance. To the general physician facts which are stated in simple terms—results which are tabulated or given in practical forms—are of more interest and of more value than more scientifically stated and deeply complicated experiments which are not readily applicable to cases at hand.

TECHNIC

The solutions used were bichloride of mercury in the strengths of 1:1000, 1:4000, 1:15,000; lysol, ½ per cent; potassium permanganate, 1:1500; and normal saline as a control. The bacteria employed were stock 24-hour cultures of staphylococcus pyogenes aureus, obtained from a case of furunculosis, B. coli of intestinal origin; and B. subtilis. B. subtilis, a spore-former, was used to determine the action of the germicides on this type of organism.

Suspensions were made of the above fresh 24-hour cultures as in preparing vaccines, and one loopful of this suspension was transferred to a test tube one-third full of sterile normal saline. A loopful of this greatly diluted bacterial suspension was then added to melted plain agar tubes, sufficiently cooled so as not to interfere with bacterial growth. These tubes were rolled in grooves on ice, the agar quickly solidifying and forming a thin, uniform film about the sides and bottom of the test tubes. Owing to the constant amount of agar and the uniform size of the tubes, the films were practically two to three millimeters in thickness and practically uniform. These tubes were placed in the incubator for 24 hours. At the end of this time discrete, superficial, and deep colonies were readily visible. The tubes were then ready for irrigation. These tubes were considered to represent the vagina; the agar representing a smooth mucous membrane covered with bacteria, and the deeper colonies representing collections of organisms in the deeper glands and crypts.

*From the Mary M. Emery Department of Pathology, University of Cincinnati.

The irrigation apparatus consisted of a four-gallon, distilled water laboratory bottle. From the outlet there extended a Y tube to the branches of which were connected, by short pieces of rubber tubing two long capillary pipettes. A similar outfit was employed which contained sterile normal saline solution. With this, the excess germicide was washed off following irrigation.

The large bottle was filled with the germicidal fluid to be used in each experiment. Two inoculated and incubated roll tubes were then placed in inclined racks. The capillary pipettes were inserted to the bottom of each test tube and the germicidal fluid allowed to run in slowly. The tubes were so placed in the racks that the fluid would rise in the tubes and keep the artificial mucous membrane constantly bathed with germicide. The flow was controlled by screw clamps applied to the rubber tubing so that fifteen minutes were required for the use of one-half pint of the solution. The fluid, after washing over the surface of the agar, rose in the tube, finally trickling out of the mouth. The force of the stream was quite sufficient to wash off many of the superficial colonies, but was not swift enough to loosen the agar. Following this irrigation for fifteen minutes, the tubes were inverted, the germicidal fluid allowed to drip out, and the tube then washed with normal saline solution in order to wash off any excess germicide on the agar. The tubes were again inverted until quite dry, when the cotton plugs were thoroughly flamed and inserted into the tubes (these plugs had been kept in sterile petri dishes during irrigation). The test tubes were again incubated for 24 hours.

In every experiment, ten roll tubes of each of the bacteria were used with each germicidal solution. Each experiment, therefore, represents the action of the germicide in sixty cultured tubes, thirty of the superficial and thirty of the deeper.

Five cubic centimeters of sterile normal saline were added to each of the irrigated tubes—these were gently agitated in order to wash off any superficial growth. In practically all instances the saline contained large floating colonies, or was clouded with bacterial suspension. This saline solution was then transferred to a plain broth medium. In order to obtain the deeper colonies, these were picked out by means of a platinum loop and broken up on the sides of other plain broth tubes. The tubes in each series were numbered from 1 to 10.

This second series was again incubated and examined at the end of 24 hours. All tubes appearing to contain a growth were now subcultured on plain agar slants; the growths were later examined microscopically. All tubes showing no growth were again incubated from one to two weeks. Those tubes which had remained sterile for 24 hours continued to remain sterile for the time of observation. This would serve to demonstrate that in these the action of the germicidal fluid was not retardative in character, but destructive.

In order to be certain that bacteria in the superficial suspensions and deeper bits of agar were transferred, the debris at the bottom of the tubes containing no growth was removed and examined microscopically and in all instances showed bacteria which had evidently been killed.

Another series of experiments was carried out practically in the same manner, with the exception that normal saline was used in the place of a germicide. In this series the bacteria were uninfluenced. This was done in order to prove

that irrigation with germicides was not mechanically eliminative in effect, but truly germicidal.

In the accompanying table the results are arranged so that the figures represent the percentage of the tubes in which the germicide was effective; i. e., bacterial death resulted. The numerator represents results on superficial growth, and the denominator, the deeper effects. It will be seen that *bichloride of mercury* in 1:1000 and 1:4000 strengths destroyed all the bacteria: B. coli, the staphylococci, and the spore-former, B. subtilis. In the 1:15,000 solution, the spore-former remained viable while the two other types of bacteria were destroyed. *Lysol*, ½ per cent strength, was about as effective as bichloride of mercury, 1:1500; that is, about 90 per cent efficient. Potassium permanganate in 1:1500 strength was remarkably without effect in any of the types. *Normal saline* did not even act as an effective mechanical eliminant.

TABULATED RESULTS OF EXPERIMENT

SOLUTION	STRENGTH	LOCATION	STAPHYLOCOCCUS	B. COLI	B. SUBTILIS
Bichloride	1:1000	superficial	100	100	100
		deep	100	100	100
Bichloride	1:4000	superficial	100	100	100
		deep	100	100	100
Bichloride	1:15,000	superficial	100	100	0
		deep	100	90	0
Lysol	½%	superficial	80	100	0
		deep	100	100	0
Potassium permanganate	1:1500	superficial	0	0	0
		deep	0	0	0
Dakin's solution		superficial	100	80	100
		deep	100	100	100
Normal saline		superficial	0	0	0
		deep	0	0	0

Figures represent the percentage of tubes wherein the germicidal action of the fluids was effective.

Here then, by means of a series of simple experiments, we see the effectiveness of germicidal douching fluids *in vitro*. Of course, the roll tube is an idealized vagina, without folds or pockets for the seclusion of microorganisms, and the two can hardly be compared. Still, if potassium permanganate is ineffective in the roll tube, it surely will have little effect in a vagina. On the other hand, bichloride in 1:1000 and 1:4000 strengths is evidently thoroughly effective in destroying all superficial growth and further penetrating agar two to three millimeters and is here similarly destructive. Bichloride in the higher dilutions (1:15,000) or lysol, ½ per cent, is apparently just as efficient for the pathogenic types of organisms. Therefore, for those who fear to use bichloride as a douche because of the possibility of bichloride poisoning, the higher dilution would seem to give just as satisfactory results. Lysol, ½ per cent, is just as effective as the last, and is decidedly less dangerous.

It would have been well to have experimented with the gonococcus in a similar manner, but, owing to the difficulties of its cultivation and its pathogenicity, it was not considered. It is only fair to say, however, that the gonococcus very

probably ranks with the nonspore-forming organisms used in the experiment and should as easily be destroyed.

Because of the present interest in Dakin's solution, this was also added to the series. This solution, from personal experience, has been far too irritating as a douching agent and will not be borne by patients. Still in the short time that it was in contact with the bacteria (15 minutes) it was as efficient as bichloride in 1:1000 strength.

CONCLUSIONS

1. Bichloride of Mercury destroyed B. coli, staphylococcus pyogenes aureus, and the spore-former, B. subtilis when employed as a germicide in the preceding experiment in low dilutions (1:1000 and 1:4000) in fifteen minutes.

2. Lysol, ½ per cent, is as effective a germicide as a 1:15,000 bichloride solution as determined by the same procedure.

3. Potassium permanganate 1:1500 strength, when used in the same manner as lysol and bichloride, is wholly ineffective.

4. The solutions used did not inhibit bacterial growth, but destroyed it wherever effective at all.

5. Washing or irrigating with normal saline will not remove all bacterial growth.

AN EXPERIMENTAL STUDY OF ROOT-FILLED TEETH: PRELIMINARY REPORT

By M. B. Cohen, M.D., West Salem, Ohio

SINCE the epochal work of Billings and Rosenow, many diseased conditions have been traced to focal infections in the oral cavity; clinicians have learned that the mouth contains something besides the tongue and are intelligently examining the teeth and the tonsils. Until recently medical students and internes were not taught to notice the condition of the gums and teeth or to associate disease in these organs with the patient's bodily ills. They were allowed to gain the impression that these organs served a mechanical purpose only, and that their pathology was of importance only in proportion to the disturbance of local function which it caused. Lately many observers have commented on the remarkable clinical results in arthritis, neuritis, myositis, and other obscure symptom-complexes following proper dental procedures.

Because it is relatively easy to diagnose a case of pyorrhea and to get the cooperation of the patient in carrying out proper methods of treatment, the relation of this disease to many systemic conditions has been established clinically.

There is another type of tooth disease, however, which, while not so spectacular in its local end results, is probably as dangerous to the health of the patient, namely, the periapical infection. Unless accompanied by a fistula, this painless condition was, until recently, undiagnosed or disregarded, as it was a common practice to regard a painless tooth as a healthy one. The routine use of the x-ray has made the diagnosis relatively easy and has instituted a study of this type of infection by many workers. It has been claimed to be possible to completely

sterilize these areas through the root canals, to completely fill the latter, and to eradicate the disease; and roentgenograms have been produced which apparently show a growth of new bone around the tooth apex.

While such results may be possible in the hands of an expert like Callahan, of Cincinnati, it is useless to expect the rank and file of dentistry to do this work, as it requires great patience, accuracy, absolute asepsis, and bacteriologic control of each stage of the operation.

That root canal filling in average hands does not eradicate the disease is shown by the following study of six cases of polyarthritis.

Sixty-two cultures were made from "locked areas" beyond the apices of eighteen teeth that had been root filled from six months to twenty years previously by sixteen different dentists, some of whom are supposed to be authorities in their profession. The following two methods for obtaining cultures were used: If the culture was made for research purposes only, the gum and alveolar process were isolated with cotton rolls like those used routinely in dentistry, dried, and painted with full strength tincture of iodine. A dental hypodermic syringe, which had been previously boiled and allowed to cool, was fitted with a needle, sterilized in the same way and finally passed through a bunsen flame. A puncture was then made through the gum and alveolar process down to the root tip, and an attempt was made to aspirate. The aspirated material, whether visible or not, was transferred to suitable culture media and taken to the laboratory for study. Care was taken to culture only those teeth which had no pyorrhea pockets surrounding them. If the tooth was to be extracted, an area for two inches around was isolated with cotton rolls and painted with iodine. The region was then infiltrated with novocaine solution which had been boiled and proved to be sterile by bacteriologic checks. The electrocautery was next used around the neck of the tooth and it was extracted through this sterile field with a forceps which had been heated in the flame. The apex was then clipped off into the culture medium by means of a heavy rongeur forceps which had also been flamed.

Growth was obtained without exception from each of the sixty-two cultures on some one of the media used. The organisms usually isolated were those found normally in the mouth. The streptococcus viridans was the predominating one; it occurred in sixty cultures. The staphylococcus family was represented in sixteen, always in combination with the streptococcus, while the colon bacillus was isolated in pure culture once. One culture yielded bacillus acidophilis.

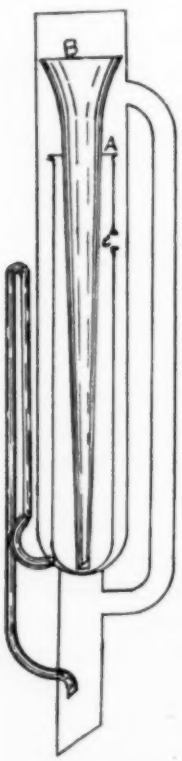
It was not possible to obtain cultures from normal teeth by extraction so that control of the second cultural method described above could not be obtained. The area beyond the apex of one normal tooth was aspirated and cultured for control purposes. This did not yield a growth.

If present day dental technic in average hands does not eradicate this type of mouth infection, it is evident that dead teeth in a patient's mouth are a source of danger. Unless an expert root canal operator is at hand, it is good practice to have such teeth removed. To obtain the best results there must be complete cooperation between the physician and the general dental practitioner that they may decide which teeth should be preserved and which teeth must be removed.

MODIFICATIONS OF THE SOXHLET EXTRACTOR*

By J. W. Weir, Oklahoma City, Okla.

THE Soxhlet extractor requires that the material to be extracted shall be a solid, and, in consequence, liquids or semisolids can be handled only after evaporation to the solid state. The described modification allows of the Soxhlet being used to extract liquids with either lighter or heavier solvents, the only requisite being that the solvent shall be immiscible. When ether or any other solvent floating on the solution being extracted is used, the modification consists of the test tube *A* having a perforation *C* blown in its side at a point higher than the upper level of the Soxhlet siphon. The material to be extracted is placed in this tube *A* and the funnel *B*, made by drawing out a test tube in the blast lamp, is placed inside of *A*. The mouth of this funnel should be large enough to insure the catching of the solvent as it drops from the condenser, but must not fit tightly within the extractor, since vapors must pass around it.



The material being extracted will remain in the bottom of test tube *A*, while the ether or other solvent passes down through *B*, and up through the material being extracted, and overflows through opening *C* into the body of the extractor. When this overflow has reached the crest of the siphon, it will be siphoned off into the lower flask, but as opening *C* is above the level of this siphon the material being extracted is not drawn into the lower flask.

If extraction is being carried on with a solvent which is heavier than the material extracted, the funnel *B* is not used, but is replaced by a second test tube fitting loosely within tube *A* and having a small hole blown in its bottom. This inner test tube must reach well towards the top of the extraction chamber. Test tube *A* is removed from the extractor and filled as far as overflow *C* with the solvent.

The bottom of this test tube is then introduced into *A* far enough that at least one or two inches of solvent rises within it. The sample is then introduced into the inner test tube and the two tubes are placed in the extractor. The solvent drops through the material being extracted in the inner test tube, passes out through the perforated bottom, into the tube *A*, overflowing at *C* and completes the siphoning into the lower flask, as in the first described modification.

For making ether extractions of milk, feces, blood, etc., these modifications of the Soxhlet are decidedly saving of time and in no way affect the accuracy of the extractions.

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EDITORIALS

Our Former Teachers in Germany

MANY medical men in this country have visited Germany for the purpose of continuing their studies. The laboratories and clinics of German universities were quite familiar to American medical men. We know the Anglo-American Societies at Berlin and Vienna and have spent many pleasant evenings at them. There we met many of our colleagues from this side and were introduced to leading German professors who talked to us and considerately offered to form classes in the various medical specialties if so many would attend at so many marks each. We learned much at some of these special clinics and lectures, and our professors were able to convert many dollars into marks. The relations between teacher and students were pleasant and for the most part profitable to both. We learned to admire many of these great teachers and possibly to love a few of them. When the war began our old teachers assumed that their old students would be pro-German. Indeed, they could conceive of nothing else. Probably no one in this country, not of German birth or descent understood the German mind and its attitude toward science and truth better than those of us who had studied in German universities. To us the German professor revealed

himself better and more fully than anyone else could have done. Every lecture and demonstration was an exhibition of his psychology and in this there were many things which no truth-respecting individual could admire. He was generally a learned man in a narrow way, but too often his learning was greatly overshadowed by his arrogance. To him science was "Die deutsche Wissenschaft" and he found frequent opportunity to extol it. He seldom referred to the researches of the men of other nations, and, when he did so, he most authoritatively criticized, minimized, or misstated the facts.

In a course of lectures on the development of abdominal surgery I heard one of the most eminent of German professors say that Bilroth was the first to do an ovariotomy. As I listened I thought of the shades of Ephraim McDowell and his immediate American and English successors, and I wondered whether ignorance or arrogance was the basis of the false statement, and I pitied my German coauditors who evidently accepted it as truth. In this instance I did dare to remonstrate with the learned man at the close of his lecture, but the interview still left me undecided between his ignorance and arrogance. A course of lectures in the history of our knowledge of digestion contained no reference to the work of our Beaumont and Dunglison, although it is true that at the very time that Beaumont was making his classical studies on Alexis St. Martin, the professor of physiology in the University of Berlin was teaching that the stomach is simply a storage and not a digestive organ. I have searched the voluminous German literature on toxins and antitoxins without finding mention of the fundamental researches of Mitchell and Reichert at the University of Pennsylvania or that of Sewall at the University of Michigan. On the other hand, Calmette and other French immunologists, on visiting Ann Arbor, have first of all wished to see the place where Sewall first immunized pigeons to snake venom. The German professor begins the history of tuberculosis with the discovery of the bacillus by Koch, and one would in no way detract from the honor due to the untiring zeal of this great man; but it is a fact that at least a decade before Koch began his work on tuberculosis, Villemin had demonstrated the presence of a virus in the tissue and certain excretions of tuberculous animals, including man. Indeed, he went much further than this and demonstrated the unity of tuberculous infections, a fact up to that time most vigorously denied by Virchow and other German teachers.

We know how the German mind has misunderstood and misinterpreted the teachings of Darwin. In the "survival of the fittest," he has decided that he is the only one "fit." "In the struggle for existence," he has shown himself capable of using the most brutal weapons and to resort to the prostitution of science to the accomplishment of his personal and national desires. His arrogance, so plainly in evidence in his classrooms years ago, has grown into a megalomania which has engulfed the world in a cataclysm and threatens to overthrow the pillars of civilization. The German mind is still in an infantile state, and science in German hands is as dangerous as explosives in a playroom.

American scientists who have studied at German universities are not as a rule pro-German. Individually we respected many of our teachers, but we were not blind to their defects. Because we did not leave the table in rudeness, they

assumed that we enjoyed the food they supplied, and we did. There was some real sustenance in the broth and a few plums in the pudding, enough to make it worth while, since we already had the fundamentals of science and were able for the most part at least to distinguish between the real and the false. In short, our German professors at that time gave us both instruction and amusement. We were not idiots, blind, or deaf, but we did not fully appreciate the pathologic significance of that well-nigh universal German attribute of arrogance and self-conceit. Even at that time it occasionally became boresome and even disgusting, but we did not fully realize its malignant capabilities. It has grown into a great tumor and must be excised if it takes the rest of the world and all time to do it.

Even the tyro in science knows that most of its great discoveries are not of German origin. What of the steam engine, the compass, the telegraph, the telephone, the aeroplane, even the submarine—the list might be indefinitely extended. Science is the exclusive property of no nation; its functions are normally beneficient; its devotees seek the welfare of the race, and not personal or national aggrandizement. Did we have patents on the medicinal uses of quinine, the iodides, the employment of anesthetics and antiseptics? No, we left the patent medicine business to charlatans in this country and German scientists abroad, and both fattened on our good nature and our dollars.

—*V. C. V.*

Culture Methods for the Isolation of B. Tuberculosis

THE isolation and pure cultivation of the tubercle bacillus has from the time of Koch's earliest discoveries been best attained after passage through a guinea pig. Contamination has been too frequent when endeavoring to procure cultures of tubercle bacilli from sputum or tissue in any other manner. A solution of this difficulty was expected in the introduction of antiformin; but this method was soon discarded by bacteriologists. In recent years efforts have been directed to discover a culture medium which would inhibit the growth of extraneous organisms, while permitting the growth of B. tuberculosis. Williams and Burdick[1] report the history of this effort.

Von Drigalski and Conradi[2] (1902) observed that crystal violet could inhibit the growth of many bacteria when added to the culture medium; but had no effect on the culture of typhoid or colon bacilli. Churchman[3] (1912) discovered that gentian violet possessed an unusual merit in such selection. By adding gentian violet (0.001%) to his culture medium, Churchman purified a culture of B. tuberculosis that had become contaminated with B. subtilis. Churchman states that B. tuberculosis is "gentian-negative," whereas B. subtilis is "gentian-positive," the effect of the gentian being described as "bacteriostatic" rather than bactericidal. Petroff[4] (1915), working with this as a basis, devised a medium composed of meat juice, glycerine, and egg, to which was added minute amounts of gentian violet. Williams and Burdick[1] (1916), among other objections, found that Petroff's medium did not contain enough moisture to prevent rapid drying. They, therefore, devised a new culture medium for the

tubercle bacillus. Williams and Burdick approved the use of sodium (hydroxide) and gentian violet as suggested by Petroff; but employed, as the substance of their medium, egg white, egg yolk, glycerine, meat infusion and agar, a medium on which Besredka had advised the growing of the bacillus for the production of an antigen suitable for complement-fixation tuberculosis tests. Some workers have, we understand, found that the medium of Williams and Burdick also has too rapid a tendency to dry out.

A. S. Griffith,[6] who had so vast an experience in the investigations of the British Royal Commission on Tuberculosis, would seem to have perfected methods of isolating the tubercle bacillus by the aid of antiformin. Some time ago Griffith found that the presence of a small quantity of antiformin in the fluid used for sowing cultures did not interfere with the growth of the tubercle bacillus. He, therefore, merely sowed successive cultures from a mixture of sputum and antiformin after varying intervals. Griffith[7] concludes from his most recent work with animal tissues, as well as with sputum, "that it is more advantageous, especially when dealing with tenacious sputum, to use high percentage of antiformin with short exposure, than low percentage with long exposure."

Griffith (quoted by Cobbett[8]) now makes a 15 per cent addition of antiformin to sputum. "The first sowing should be made one or two minutes after mixture, according to the consistency of the sputum, and three tubes at least ought to be sown within the first five minutes. Thereafter the intervals may be longer—about five minutes, especially if the sputum remains undissolved and mucinous. There is no need to sow tubes after twenty minutes or after the sputum has completely dissolved."

BIBLIOGRAPHY

[1]Williams and Burdick: Jour. Bacteriol., July, 1916, i, No. 4.
[2]Von Drigalski and Conradi: Ztschr. f. Hyg. u. Infectionskrankh., 1902, xxxix, 283.
[3]Churchman, J. W.: Jour. Exper. Med., 1912, xvi, 221.
[4]Petroff, S. A.: Jour. Exper. Med., 1915, xxi, 38.
[5]Besredka, A.: Compt. rend. Acad. d. sc., clvi, 1633.
[6]Griffith, A. S.: Brit. Med. Jour., 1914, i, 1171.
[7]Griffith, A. S.: Lancet, London, 1916, i, 723.
[8]Cobbett: The Causes of Tuberculosis, Cambridge, 1917.

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